June 20, 2002

8:30 am	Soft Tissue Cytopathology –Diagnostic Hazards & Expectations *P. Wakely, Jr.*
9:15 am Lymphoma	The Role of FNA in the Evaluation of Malignant *P. Wakely, Jr.*
10:00 am	Advances in the Pathobiology of CMV Infection in Renal Transplant Patients *D. Sedmak*
10:45 am	COFFEE BREAK
11:15-am	The Role of Ultrastructural Examination in Fibroblastic/Myofibroblastic Lesions *B. Eyden*
12:00 am	Surgical Pathology –Session IV: Kidney & Adrenal. Case Presentations (4 cases) *AMR Seminar Club Members*
1:20 pm	LUNCH
3:00 pm	Surgical Pathology –Session V: Head & Neck. CasePresentations (5 cases) *AMR Seminar Club Members*
4:40 pm	COFFEE BREAK
5:10 pm	Surgical Pathology –Session VI: Hematolymphoid tissue. Case Presentations (4 cases) *AMR Seminar Club Members*
6:30-7:30	Quiz Cases (Illustrated Exhibition)

SOFT TISSUE CYTOPATHOLOGY – DIAGNOSTIC HAZARDS AND EXPECTATIONS

P. E. Wakely, Jr., M.D.
The Ohio State U. College of Medicine
Columbus, OH/ USA

I. INTRODUCTION

Soft tissue tumors are uncommon lesions. Sarcomas comprise about 1% of all adult and 15% of all pediatric malignancies. The use of fine needle aspiration(**FNA**) biopsy as a method to diagnose soft tissue tumors, in particular sarcomas, is controversial with a range of opinions. Some physicians believe incisional biopsy is required for primary diagnosis, some that FNA alone may be sufficient, and others view FNA as only useful for locally recurrent and metastatic disease. Interpretation of FNA smears from somatic soft tissue can be extremely demanding.

Reasons for Diagnostic Difficulty in Soft Tissue FNA:

- Relative infrequency of soft tissue tumors(except lipoma).
  -Pathologists less familiar and thus less "comfortable" with such lesions.
  -Most clinicians do NOT perform or will not consider FNA for a patient with a soft tissue mass.
- Overlapping morphologic features of many tumor(especially spindle cell neoplasms).
- Heterogeneity of architectural patterns and/or individual cell morphology within some soft tissue tumors. Proper sampling becomes a major issue in some lesions with different features in different areas.
  -Spatial relationships of different morphologies often lost.
  -Limited cellularity in fibrous lesions may preclude sampling of adequate/diagnostic cells.

Even though there are many benefits to soft tissue FNA as listed in the box to the right, this is far from being an infallible procedure. The major benefit of first attempting soft tissue FNA is the quick, cheap, and harmless nature of a test that will in most instances produce a result that will allow the clinician to proceed to a more informed procedure. An example would be the patient who is thought to instead to harbor an abscess.

♦ Soft Tissue FNA - Benefits

-Rapid Turnaround Time
-Facilitates Triage of Patient and Specimen
-Cost-effective
-Relatively Painless. Anesthetic unnecessary. Office procedure.
-Single FNA Has Greater Sampling than Single Core Biopsy
-Complications: rare
-Does not contaminate a subsequent surgical site.
-Confirms a metastatic/recurrent lesion in a known cancer patient.

Accuracy of soft tissue FNA is dependent on many factors:

- Clinical setting in which FNA is practiced: pathologist performed FNA vs. clinician performed; slides submitted with minimal to no clinical/radiographic history vs. pathologist having full access to medical and radiologic records.

to the diagnosis, it can also hinder one's ability to sufficiently characterize the lesion. The polymorphism that can exist from one field to the next in some soft tissue tumors is largely unappreciated in smears.

In addition, FNA does not allow for the determination of a capsule, and if present whether the capsule completely or partially surrounds the lesion. This also means that one cannot tell whether a tumor has an irregular infiltrative border, or whether it is a neoplasm with a smooth "pushing" type of border.

The vascular pattern that is characteristic or helpful in certain soft tissue lesions is also lost in smears. Mitotic activity is important in evaluation of tissue sections of soft tissue tumors, but is nearly always underestimated in smears because of the marked difference in which one looks at the same thing. With smears, one is looking at a more or less monolayer of cells, while with paraffin wax sections one is looking at multiple layers of cells stacked on top of one another in which a 5 μm. slice is laid onto the glass.

Nonetheless, even with these limitations, the expectation and existing evidence is that in those centers with soft tissue FNA experience this technique can separate benign from malignant lesions, and can accurately subclassify lesions into clinically relevant groups that permit practical patient management. These groups and some of the entities from these groups are discussed further.

II. SPINDLE CELL DOMINANT LESIONS

In the category of spindle cell lesions the major challenge is differentiation of a benign spindle cell lesion from a spindle cell sarcoma. The two principal cytologic features used are cellularity and individual cell morphology.

A. Fibromatosis

- many histologic subtypes – not usually distinguishable from one another by cytologic morphology
- *hypocellular smears*: cells embedded in collagenous stroma difficult to extract by FNA
- spindle, stellate cells in loose clusters or as single forms
- oval to elongated smooth contoured nuclei, small nucleoli; uniformly sized fibroblasts
- thin delicate/wispy cytoplasmic tendrils – unipolar and bipolar
- collagenous stroma
- entrapped atrophic myofibers with clustered nuclei.

B. Benign Nerve Sheath Tumors(Neurofibroma and Schwannoma)

◊ smears from both lesions are very similar; often indistinguishable from each other
◊ patient may experience sharp stinging radiating pain when these tumor is aspirated - a good clinical clue
◊ spindle cells in loose clusters and single forms
◊ often but not invariable distinctive irregular nuclear outline described as "buckled", "wavy", or "fish-hook"
◊ cytoplasm pale, in thin strands; cell borders typically indistinct
◊ isolated nucleomegaly and pleomorphism possible in schwannoma
◊ parallel palisading groups(Verocay bodies) uncommonly seen in aspirates of schwannoma
◊ metachromatic stroma is variable; can dominate smear in myxoid neurofibroma.

C. Synovial Sarcoma **(SS)**

On a case by case basis, most spindle cell sarcomas cannot be separated from one another at the time of initial diagnosis without accompanying ancillary immunophenotyping or electron microscopy. In contrast, aspiration cytopathology is generally an excellent method for confirming specific recurrent and/or metastatic spindle cell sarcomas. The paradigm tumor in the FNA morphology of spindle cell sarcomas is synovial sarcoma.

- biphasic type of SS (gland formation) difficult to appreciate on smears
- rich cell yield dispersed as single cells or in thick tightly aggregated clusters with multiple cell layers
- relatively **uniform** spindle shaped cells; oval or oblong monotonous nuclei; minimal anisonucleosis
- glandular(epithelial) component: acinar arrangement of cells with nuclear palisading
- cytoplasm is scant and often stripped from nucleus.
- mitoses variable, often common.
- generally absence of necrosis. Minimal background stroma.

Other Spindle Cell Sarcomas: Leiomyosarcoma(LMS)/Fibrosarcoma/Malignant Peripheral Nerve Sheath Tumor(MPNST)

From a purely morphologic standpoint, these various sarcomas closely mimic monophasic SS and without immunophenotyping cannot be differentiated from one another. This statement applies to both aspirate smears, core biopsies, and resection specimens.

- the 2 principal cytologic features of spindle cell sarcoma are: marked hypercellularity of smears, and intense nuclear chromasia of spindle cells with or without nuclear atypia.
- smears of poorly-differentiated LMS and MPNST differ from SS and fibrosarcoma in that a more or less isomorphic population of spindle cells is replaced by a greater degree of anisonucleosis, and nucleolomegaly. Well-differentiated forms of LMS and MPNST are similar to SS.
- smear background usually clean, but necrosis is common in less differentiated tumors
- mitotic figures more likely to be seen in poorly-differentiated spindle cell sarcomas

III. LIPID RICH SMEARS

Lipoma

- the most common mesenchymal tumor of man; superficial or deep seated
- the mature fat cell is spherical with a large, single cytoplasmic vacuole and a thin discrete cell border
- the nucleus, placed at the cell edge, is small, rounded or slightly angulated. When viewed on end it appears flattened and crescentic.

The diagnosis of Lipoma has to be inferred by the presence of a mass, because FNA shows mature lipocytes, but cannot demonstrate the fibrous capsule that is typical of lipoma. Identical cytomorphology is seen in aspirates of normal subcutaneous tissue or breast.

Fat Necrosis

¶ often occurs in association with mesenchymal repair

¶ pure fat necrosis, in my experience, is more often seen in aspirates from the breast rather than the subcutaneous tissue.

¶ moderately cellular smears

¶ lipocytes with single as well as multivacuolated cytoplasm

¶ multinucleated giant cells, granular background cellular debris, macrophages, and inflammatory cells

Pleomorphic Lipoma (**PL**)

- pseudosarcomatous lesion often mistaken for liposarcoma
- generally arises in the subcutis from the back of the neck, shoulder, or facial region
- middle-aged or older males
- large, pleomorphic cells admixed in adipose tissue is reminiscent of pleomorphic liposarcoma
- pleomorphic single and multinucleated cells; latter are so-called "floret" cells because nuclei are placed in a peripheral circular arrangement around the cytoplasm analogous to the petals of a flower
- absence of necrosis, and mitotic figures.

Pitfall: The individual cell pleomorphism of PL can fool one into issuing a malignant diagnosis. Always correlate with clinical features. PL is nearly always in a superficial subcutaneous location in the head and neck and shoulder region – a location that is distinctly unusual for the vast majority of pleomorphic sarcomas.

Myxoid Liposarcoma

- most common subtype of liposarcoma (**LPS**)
- distinctive cytomorphology – 3 criteria for diagnosis:

1.) ramifying delicate capillaries producing so-called "chicken-wire" appearance in a,

2.) myxoid "metachromatic staining" stroma more often distributed as discrete fragments rather than a "film" smeared across the slides and,

3.) univacuolated lipoblasts, usually signet ring type of morphology but occasional multivacuolated lipoblasts with nuclear scalloping.

- myxoid and round cell types often intermixed; all FNA diagnoses of Myxoid LPS – a low grade sarcoma – must be made with the knowledge that a component of Round Cell LPS – an intermediate grade sarcoma – cannot be excluded due to potential sampling error

Other Liposarcoma Types

- pleomorphic LPS has features that mimic those listed for any pleomorphic sarcoma
- lipid differentiation not always visible in smears of Pleomorphic LPS
- Well-Differentiated LPS smears are only modestly cellular, while other subtypes generally show high cellularity.
- dominance of mature lipocytes in W-D LPS; cytoplasm varies from large single vacuoles with eccentric nuclei to coarse multiple vacuoles
- vascularity much less in round cell and W-D LPS

♦ Round Cell LPS can mimic a malignant small round cell tumor
♦ background clean except for abundant metachromatic stroma in myxoid LPS

IV. EPITHELIOID CELL DOMINANT SMEARS

Granular Cell Tumor (GCT)

∫ arises in muscle, subcutaneous tissue, as well as mucous membranes
∫ moderately cellular smears
∫ polygonal/epithelioid shaped cells in loose syncytia and single cells
∫ KEY FEATURE: recognizing the fine to coarse cytoplasmic granularity; better seen in Papanicolaou stained smears
∫ nuclei spherical to oval; nucleoli may be obvious; bare nuclei common
∫ indistinct, never sharply demarcated cell borders

Adult Rhabdomyoma

• generally occurs in the head & neck region including the pharynx – a similar areas as granular cell tumor
• single cells and cells in small groups
• round nuclei, single nucleoli, and abundant cytoplasm
• KEY FEATURE: finely granular cytoplasm, orderly linear cross-striations seen best in Papanicolaou stained smears
• clean smear background

Alveolar Soft Part Sarcoma (ASPS)

∇ occurs in older teenagers and young adults
∇ sites: head and neck - children, thigh – adults; similar to granular cell tumor
∇ nested(alveolar) architecture seen in tissue is rarely appreciated on smears
∇ large monotonous cells; variable cellularity, often bloody with cell dispersion
∇ central and eccentric rounded nuclei with smooth contours, occasional binucleation; cytoplasmic fragility leads to many bare nuclei
∇ single large prominent nucleoli
∇ cytoplasm abundant, finely reticulated with some cells showing faint striations
∇ mitoses, background stroma, and necrosis rare
∇ delicate light striations within cytoplasm may represent the characteristic membrane bound crystals with rectangular or rhomboid shapes found ultrastructurally

Giant Cell Tumor of Tendon Sheath (Localized Nodular Tenosynovitis)

• forms a mass usually on the hands(fingers and palm), feet or around the knee
• KEY FEATURE: presence of numerous osteoclast-type multinucleated giant cells along with the single polygonal cells.
• cellularity - moderate to high .
• polygonal cells singly dispersed; many binucleated; moderate, minimally granular cytoplasm with sharp cell borders
• nuclei: uniform, eccentric, round to oval, fine chromatin; indistinct nucleoli
• hemosiderin laden macrophages - variable.

Clear Cell Sarcoma (CCS), aka Melanoma of Soft Parts

♣ neoplasm of young adults; one-fifth of patient are < 20 years of age
♣ soft tissue of extremities, notably the foot and knee are most often affected
♣ of neural crest derivation, and shares many histochemical, ultrastructural, and cytogenetic features of malignant melanoma including melanin, melanosomes, and premelanosomes
♣ very aggressive neoplasm with a poor prognosis
♣ tumor size >5 cm. and the presence of necrosis stand out as poor prognostic features
♣ aspirates highly cellular with cells scattered singly and in loose groups
♣ relatively monotonous oval, rounded intermediate-sized cells
♣ nuclei often eccentric with a single nucleolus
♣ moderate finely granular and pale cytoplasm with well-defined cell borders
♣ occasional mitoses and multinucleated giant cells.

Metastatic Malignant Melanoma

◘ mimics almost the entire spectrum of aspiration cytology in soft tissue aspirates including spindle cell, epithelioid, and pleomorphic tumors
◘ smears are highly cellular; cells are more often individually dispersed, but can also appear in aggregates
◘ cell shapes vary from fusiform, to polygonal, to rounded, to pleomorphic; most rounded
◘ KEY FEATURE: binucleated mirror image nuclei and intranuclear inclusions are characteristic(but not pathognomonic of) melanoma
◘ nucleoli are usually rounded and obvious, but can be huge and bizarrely shaped
◘ cytoplasm typically abundant and may be vacuolated thus mimicking MFH and pleomorphic liposarcoma
◘ amount of pigment extremely variable – often present in only 50% of cases: varies from a fine dusting of cell cytoplasm to large chunks of intra- and extra-cytoplasmic pigment

Extramedullary Plasmacytoma

⊗ plasma cell dyscrasias have a spectrum of clinical presentation from localized indolent, soft tissue masses to aggressive multiple myeloma
⊗ FNA of an extramedullary plasma cell tumor may be the first indication of a previously unsuspected myeloma.
⊗ aspirates variably cellular with pattern of singly dispersed, non-cohesive cells
⊗ small to intermediate sized rounded to oval cells with moderate amount of cytoplasm; prominent perinuclear area of cytoplasmic clearing
⊗ eccentrically placed nuclei; binucleation and multinucleation common; clumped nuclear chromatin, and smooth nuclear membranes; nucleoli variable in size, often indistinct
⊗ background lymphoglandular bodies usually scant.

V. SMALL ROUND CELL DOMINANT SMEARS

Lymphangioma

- more common in infants and children, but occasionally seen in adults
- aspirates yield translucent, straw-colored fluid unless contaminated by blood – best to submit most of the fluid for cytocentrifugation rather than making many smears
- cell number is variable, and consists almost entirely of small, mature lymphocytes. Some monocytes and macrophages may be seen.
- an amorphous proteinaceous background also common

Rhabdomyosarcoma (RMS)

- most common primary soft tissue cancer in children
- various histologic subtypes of RMS(embryonal, classic alveolar, solid alveolar) not distinguishable on smears
- embryonal RMS most likely to occur in the head/neck region; alveolar RMS favors extremities of older children and adolescents.
- hypercellular aspirates with singly dispersed cells and cells in crowded aggregates
- small to intermediate sized cells; rounded nuclei with very high N/C ratio in undifferentiated cells
- oval nuclei, minimally pleomorphic, hyperchromatic; dense chromatin; variable nucleoli
- differentiating rhabdomyoblasts: more often have 2 or more nuclei per cell; more often have a moderate amount of cytoplasm unlike meager cytoplasm of undifferentiated cells
- cytoplasmic cross striations exceedingly rare
- mitoses and cell necrosis variable.

Most aspirates of RMS fall into the small round cell category rather than that of a spindle cell neoplasm. *Differential diagnosis includes metastatic neuroblastoma, extraosseous Ewing's sarcoma, desmoplastic small round cell tumor, and non-Hodgkin's lymphoma.*

Extraosseous Ewing's Sarcoma/PNET family

- chiefly arises in soft tissues of the paravertebral region, retroperitoneum, chest wall, and extremities
- in nearly all cases of "malignant small round cell tumors of childhood" [of which Ewing Sarcoma is the paradigm] a menu of antibodies should be performed; cytogenetic and molecular analysis may also be required
- highly cellular aspirates; single cells with a minority in loose clusters; rare pseudorosette formation
- monotonous uniform cells(2-3x the size of mature lymphocytes)
- single round to oval nuclei, indistinct nucleoli; bare nuclei common
- bi-nucleation & multinucleated cells are rare
- minimal visible cytoplasm, cytoplasmic vacuoles or blebs variable; occasionally prominent.
- background stroma absent.

Ultrastructurally PNET contains few dense-core neurosecretory granules and cell processes supportive of a neuroectodermal origin. Average age is 15-30 years, x= 14 years. Most patients are female. Local recurrence is common

PNET is currently placed at one end of the continuum in the Ewing's sarcoma complex. PNET differs from classic neuroblastoma in that it is catecholamine negative, contains HLA antigens, generally occurs in adolescents - not young children, and produces stromal collagen. It share an identical chromosome abnormality t(11:22)(q24;q12) with Ewing's sarcoma which is currently considered to represent the other(undifferentiated) end of this spectrum.

VI. PLEOMORPHIC CELL DOMINANT SMEARS

Malignant Fibrous Histiocytoma (MFH)

- surpasses all other primary soft tissue malignancies in frequency in adults accounting for about 25% of cases
- may arise anywhere in soft tissue with the lower extremity being the most common site
- cytologically indistinguishable from other pleomorphic forms of sarcoma, e.g. leiomyosarcoma, liposarcoma, MPNST, and RMS
- smear pattern: single cells and loose clusters
- marked variation in cell size/shape, nuclear size/shape
- most nuclei are large, rounded or irregular; others are spindle and stellate shaped
- macronucleoli common, intranuclear pseudoinclusions
- cytoplasm may be voluminous; slight vacuolization
- background necrosis not uncommon.

VII. MYXOID DOMINANT SMEARS

Entities in this category generally possess a ground substance that seems to dominate the smear - particularly those smears which are air -dried, Romanowsky stained.

Intramuscular Myxoma

- deep, intramuscular lesion that may grow to a large size(10 cm.).
- the material expressed onto slides is generally clear and viscous with a tendency of the material to plug the needle
- air-dried smears show few cells - cytologically bland - dispersed in an abundant background stroma that stains metachromatically in Romanowsky preparations
- matrix is semi-transparent rather than opaque
- cells vary from plump, rounded forms to slender spindle or stellate shaped structures.

Soft Tissue Ganglion

- distinction from intramuscular myxoma based primarily on location not morphology
- both lesions cytologically very similar
- cellular material is limited to vacuolated macrophages

Myxofibrosarcoma (Myxoid Malignant Fibrous Histiocytoma)

- adults >50 years

Rhabdomyosarcoma	-/+	++	-/+	-	-/+	-	-
Liposarcoma	-	-	-/+	-	-	-	-
Clear Cell Sarcoma	-	-	++	+/-	-	-	-
Ewing's Sarcoma	-/+	-	-	-	++	-	-
Chordoma	++	-	++	++	-	-	-
ASPS	-	-/+	-	-	-/+	-	-
Melanoma	-	-	++	-	-	-	-
Epithelioid Sarcoma	++	-	-	++	-	+	-

++, strongly positive in most cases; +, weakly positive; -/+, focal positivity on occasion; -, usually negative.

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QUESTIONS

1. FNA features of myxoid liposarcoma include all but one of the following:
   - **A.** Discrete microfragments.
   - **B.** Branching capillary network.
   - **C.** Pleomorphic lipoblasts.
   - **D.** Metachromatic staining background.
2. The key to separating granular cell tumor from alveolar soft part sarcoma on FNA smears is:
   - **A.** Degree of nuclear pleomorphism.
   - **B.** Cytoplasmic granularity.
   - **C.** Single cell distribution versus cells in clusters
   - **D.** Cytoplasmic vacuolization.

***Mark the appropriate letter.**

1. Nella aspirazione con ago sottile del liposarcoma mixoide, tutti i seguenti reperti possono osservarsi, eccetto uno:
   - **A.** Discreti microframmenti.
   - **B.** Rete capillare ramificata.
   - **C.** Lifoblasti pleomorfi.
   - **D.** Colorazione metacromatica dello sfondo.
2. Il "clou" per differenziare il tumore a cellule granulose dal sarcoma alveolare delle parti molli su strisci da citoaspirazione con ago sottile è:
   - **A.** Grado di pleomorfismo nucleare.
   - **B.** Granularità citplasmatica.
   - **C.** La distribuzione a cellule isolate contro quella a cellule in aggregati.
   - **D.** Vacuolizzazione citoplasmatica.

***Contrassegnare la lettera giusta.**

- moderately cellular smears; occasional cells show marked nucleomegaly and pleomorphism
- myxoid stroma "smeared" in background
- minimal vascular network

Extraskeletal Myxoid Chondrosarcoma

- patients are 35-60 years
- slow-growing, but can be aggressive
- multilobulated pattern present in tissue not seen in smears
- thin to opaque myxoid stroma
- cells in linear cords or flat aggregates
- isomorphic cells; round to oval nuclei; small indistinct nucleoli; moderate amount of cytoplasm – sometimes finely vacuolated

Chordoma

- neoplasm of bone[arises only along the spinal axis as a result of its derivation from remnants of the developing notochord] that often presents as a soft tissue mass
- primary site: sacrococcyx; may present as a buttock mass; rarely occurs in head and neck aspirates
- patients in 40's to 60's.
- cells in loose groups, sometimes trabecular arrangement; also spread singly.
- large polygonal cells: mononuclear and binucleated; marked anisonucleosis. 2nd population of smaller cells with modest amount of cytoplasm.
- nuclei: rounded or oval/ intranuclear inclusions/ nucleoli obvious
- cytoplasm: voluminous/ coarse, multiple vacuoles/ physaliphorous cell
- abundant opaque & fibrillar metachromatic stroma; often intersects between cells.
- unlike its major diagnostic source of confusion - myxoid chondrosarcoma - cells of chordoma strongly express cytokeratin, and epithelial membrane antigen; both tumors stain for S-100.

Immunophenotyping of Soft Tissue Tumor Aspirates

TUMOR	Pan-cytoK	MYOGENIC MARKERS	S100	EMA	CD99	CD 34	CD31
Fibrosarcoma	-	-	-	-	-	-	-
MFH	-/+	+/-	-	-/+	-/+	-	-
Synovial Sarcoma	++	-	-	++	+	-	-
Leiomyosarcoma	-/+	+ to ++	-/+	-/+	-/+	-	-
MPNST	-/+	-	++	+	-	-	-
Angiosarcoma	-/+	+/-	-/+	-	-	++	++
Neurofibroma	-	-	++	-	-	-	-

ROLE OF FNA IN THE DIAGNOSIS OF MALIGNANT LYMPHOMA

P. E. Wakely, Jr., M.D.
The Ohio State U. College of Medicine
Columbus, OH/ USA

I. INTRODUCTION

Lymph nodes afford an excellent opportunity for diagnostic evaluation using the fine needle aspiration(FNA) biopsy technique. When compared with excisional biopsy there are several differences as listed in the table below which can make this a challenging endeavor.

Lymph Node - Surgical Biopsy vs. Fine Needle Aspiration Biopsy

	Excisional Surgical Biopsy	FNAB
•Material obtained	Tissue	Cells
•Cost	Relatively expensive	Much less expensive
•General anesthesia	Sometimes necessary	Unnecessary
•Equipment needed	Variable, can be extensive	Minimal
•Sampling error	Rarely a problem	Always a possibility
•Complications	Uncommon	Rare
•Scar/Sutures	Normal consequence	Never occurs
•Pathologist confidence	Expertise widespread	Expertise limited
•Insufficient diagnostic material	Almost never	Not uncommon; technique dependent
•Immunophenotyping/molecular studies of pathologic material	Possible	Possible

BENEFITS OF LYMPH NODE ASPIRATION

§ Triage of Patient with Lymphadenopathy

- Confirms that the mass is lymphoid tissue.
- Can preselect those patients without a prior medical history of cancer that would require surgery(e.g. Hodgkin lymphoma) from those where it can be avoided(reactive hyperplasia, some non-Hodgkin lymphomas, many infectious conditions, metastatic tumor).
- May help to focus laboratory testing for clinician thus resulting in a more informed and economical workup(e.g. granulomatous disease).
- May suggest a primary site if metastatic tumor is found.
- Provides material for culture if infectious process suspected.

BENEFITS OF LYMPH NODE ASPIRATION

§ Effective Diagnostic Tool

- Rapid turnaround time(minutes for a preliminary interpretation).
- High diagnostic sensitivity and specificity for experienced observers.
- Ability to sample multiple nodes if necessary.
- Minimal trauma. Complications rare.
- Low cost.
- Capable of obtaining cells for immunotyping, and other ancillary tests.

Some of the detractors of lymph node aspiration cytopathology continue to point to its inability to produce results equal to those obtained with tissue pathology. In most clinical scenarios, however, the

clinician is just trying to find out what has produced this mass, and therefore has a clinical diagnosis of "rule out malignant neoplasm".

BENEFITS OF LYMPH NODE ASPIRATION

§ Efficacious in the Cancer Patient

- Preserves lymph node architecture if surgical biopsy is required.
- Documents metastasis in a known cancer patient.
- Can confirm recurrence or transformation to a higher grade lymphoma in a patient with known malignant lymphoma.
- Helps in staging of tumor.

Therefore, a more realistic comparison is not between the cytologic smear diagnosis and tissue diagnosis, rather it is *between the smear diagnosis and clinical diagnosis.*

It remains true that in many instances the tissue diagnosis remains the "gold standard", but in some diseases FNA cytopathology is as good as tissue diagnosis, and in some instances a superior substitute.

Smears can be evaluated in a matter of minutes to determine whether diagnostic material is present before the patient leaves the room. This also means that the clinician can be informed of the nature of the disease process shortly after an FNA, thus allowing him/her to reduce the anxiety of patients and/or parents(which often exists in these situations) if the condition is benign, or to convince them of immediate therapy or further procedures if it is not.

Major contraindication to FNA of a superficial lymph node: a severe coagulation disorder. Even this is only a relative contraindication, and FNA may be attempted if appropriate blood products temporarily correct the situation. Hematoma formation is the only "common" complication of superficial FNA. Extremely unusual complications:

- hemorrhage, fibrosis, and partial or total infarction of lymph nodes
- FNA rarely precludes histologic analysis if the node is subsequently excised.

Caution: **One must be extremely careful when aspirating a deep axillary, low cervical, or supraclavicular lymph node because the possibility of pneumothorax(admittedly low) does exist**. Parenthetically, pneumothorax is always a risk whenever deep FNA of enlarged mediastinal lymph nodes or a mediastinal mass is attempted.

Even with all these positive attributes, lymph node FNA remains an imperfect test.

Major Shortcomings of Lymph Node FNA:

- **Sampling Error** secondary to [a] improper technique, [b] partial lymph node involvement [c] partial/complete fibrosis of a node such as can develop with Hodgkin lymphoma, or a sclerosing mediastinal non-Hodgkin lymphoma.

When fibrosis prevents the extraction of diagnostic cells from their collagen matrix one is left with hypocellular smears with few if any diagnostic cells.

- **Inability to Evaluate Lymph Node Architecture**. This can hamper the recognition of specific lymphadenopathies where evaluation of spatial relationships is required.
- **Pathologist "Comfort Level".** Because the amount of time spent in residency training in cytopathology(in North America) is much less compared to the amount spent in surgical pathology, confidence in FNA diagnoses among many pathologists is less than optimal. Thus, it is much easier for many pathologists to suggest examination of the enlarged lymph node after it is surgically excised.

LYMPH NODE FNA - LIMITATIONS

§ Sampling error secondary to:
- Improper/poor technique.
- Lymph node fibrosis
- Excessive necrosis, inflammation, or blood.
- Partial involvement of lymph node by the lesion.
- Small or deep seated lymph node.
- Lymph node/mass too large.
- Failure to obtain cells for ancillary studies, e.g. immunophenotyping, culture, molecular techniques.

§ Inability to evaluate Architecture/Vascular pattern
- Examples: Progressive Transformation of Germinal Centers, Vascular Transformation of lymph node sinuses, etc.
- Subtyping of some lymphoid disorders not possible

§ Interpretation error:
- Limited experience/expertise.
- Attempting to make specific diagnoses on limited or poorly preserved material.

- FNA is not meant to replace clinical judgment.
- Because of aforementioned potential sources of error, one should always be cognizant that *a negative result does not unequivocally mean the absence of disease*; any lymph node that is clinically suspicious, but cytopathologically interpreted as benign requires further evaluation and probably surgical excision.
- FNA, though very useful in a patient with a discrete mass, is rarely informative when performed on patients with only a vague "swelling" or induration of an area.

Before the role of FNAB in lymphoma diagnosis is discussed one must be aware of the normal microanatomy of the lymph node. The greatest difficulty in aspiration and imprint cytopathology of lymph nodes is the distinction of reactive lymphoid hyperplasia**(RLH)** from a lymphoproliferative neoplasm. A major feature used in histopathology to clarify this problem - tissue architecture - is missing from smears, thereby exacerbating a challenging problem. Since classification by architectural pattern is not possible, the principal morphologic parameter used is cell size and smear composition as major discriminators. If one examines the components of the hyperplastic lymph node histologically one finds a variety of cells occupying different parts of the node.

LYMPH NODE MICROANATOMY

Area	**Predominant Cell**
Cortex	Small(B) lymphocyte
	Follicular dendritic cells(FDC)
	Follicular center cells – centroblasts and centrocytes
	Tingible body macrophages(TBM)
Paracortex	Small(T) lymphocyte
	Interdigitating reticulum cells/Langerhans' cells
	Immunoblasts
Medulla	Plasmacytoid lymphocytes
	Plasma cells
	Immunoblasts
Miscellaneous	Capillaries, endothelial cells, mast cells, eosinophils

Back and forth excursions of FNA randomly capture lymphocytic and non-lymphocytic cellular elements from different regions, commingle them within the barrel of the needle, and then expel them as a disordered mixture onto the slide.

Two basic tenets used in recognizing cells as lymphoid on a smear[regardless of whether they are benign or malignant] are:

- *a.)* cell distribution predominantly as non-clustered, individual cells(single cell pattern), and
- *b.)* the presence of isolated globular or flake-like cytoplasmic fragments **[Lymphoglandular Bodies, a.k.a. lymphoid globules]** in the smear background.

- Little stroma in reactive hyperplasia ⇒ moderately to highly cellular smears.
- Degree of cellularity has no direct correlation with the benign or malignant nature of lymphoid cells on a smear.
- An exception to the single cell architecture in reactive hyperplasia is the presence of follicular center(**FC**) fragments, and lymphohistiocytic(**L&H**) aggregates.
- FC fragments are microscopic bits of the germinal center captured by the fine needle and expelled intact as a loose syncytium of small lymphocytes, tingible-body macrophages(**TBM**), and FC cells held together by follicular dendritic cells(**FDC**).
- FDCs: hypochromic oval nuclei, inconspicuous nucleoli, may be binucleated, often obscured by lymphocytes in an FC fragment.
- Short segments of capillaries sometimes exhibit branching in these FC fragments.
- Histiocytes may intermingle with lymphocytes and dendritic cells may also commingle with lymphocytes to produce L&H aggregates. Unlike FC fragments, L&H aggregates lack TBMs and capillary segments.
- TBMs may be seen in reactive follicular hyperplasia, high grade non-Hodgkin lymphoma, Hodgkin's lymphoma, lymph node metastases, and partial node replacement by a lesion.

II. NON-HODGKIN LYMPHOMA (NHL)

Application of FNA to lymph nodes is relatively well accepted for: infectious conditions, reative lymphoid hyperplasia, and metastatic carcinoma. When applied to the diagnosis of malignant lymphoma(**ML**) - particularly a primary diagnosis of ML in a new patient - however, opinion on the efficacy of FNA is markedly divided. Some prominent pathologists feel that nearly all non-Hodgkin lymphomas can be diagnosed with the technique, while others feel that the primary diagnosis of ML should only be based on a tissue section, never an aspirate smear alone.

Possible reasons for this controversy include:

-most cytopathologists are not hematopathologists and vice-versa.
-difficult to apply the various previous lymphoma classifications to smears.
-wide spectrum in degree of diagnostic difficulty among the various lymphoma subtypes.
-expertise widely variable.
-issue of adequacy may be challenged(and thus legal implication regarding standard of care) if outcome does not conform to a preconceived result
-political "turf" issue between hematopathology and cytopathology(e.g. control of flow cytometry laboratory)

Acquisition of enough cells is paramount not only for morphologic analysis of the smear, but for ancillary studies. The principal adjunct test required of all FNAs presumptive of NHL is immunologic phenotyping. Accomplished either with:

- flow cytometry [which is probably the most popular and preferred technique because of the large number of antibodies that can be applied to the specimen if enough cells are present],
- cytospin slides, or
- preparation of a cell block with immunohistochemical staining identical to that performed on tissue sections

Note: It has become clear that (with few exceptions) immunophenotyping is essential if accurate subtyping of non-Hodgkin lymphoma is expected from FNA biopsy.

The major classification used for ML in the past two decades in North America(*Working Formulation*) has been a major hindrance to subtyping ML using FNA because it was based to a large degree on evaluation of the tumor pattern within the node(follicular, diffuse, sinusoidal).

Note: FNA cytopathology will not recognize a specific architectural pattern of lymphoma [follicular, diffuse, paracortical, sinusoidal, capsular/extranodal invasion, partial involvement] within a lymph node.

The 2001 WHO Classification of Lymphoid Neoplasms classification should be greeted with enthusiasm by those practicing FNA. *It markedly de-emphasizes the importance of architectural pattern/spatial relationships within the node.* It proposes that ML is multiple diseases, not a single disease, and focuses primarily on the cell type[morphology, immunophenotype, genotype] in combination with the clinical features to classify each lymphoma.

Accuracy of Lymphoma Diagnosis by FNA

Oncologists expect that once a diagnosis of ML is made, it should be accurately classified. Since entire lymph nodes can be removed with little morbidity in most circumstances, the surgical pathologist generally has a large amount of tissue to study histologically, to submit for flow cytometry or genetic analysis, and to perform a battery of stains in order to correctly subclassify a lymphoma. If one is to strongly advocate for FNA to replace this time tested method, then the FNA community will have to acquire the knowledge and use the tools for proper classification.

Published accuracy of lymphoma diagnosis by FNA has varied greatly in the past, in part because many authors did not use immunophenotyping to assist them in subclassification. Some centers/pathologists use FNA as a *screening* test for lymphoma diagnosis. If this is the intent, then once a cytologic diagnosis of "suspicious for lymphoma", or "atypical lymphocytes, possible lymphoma", or even "malignant lymphoma" has been issued one's diagnostic obligation has been fulfilled. A "true" diagnosis of lymphoma with correct lymphoma subtype would have to wait until after the lymph node has been excised(which should be the next step in management).

On the other hand, if one is to use FNA as a *diagnostic* test for lymphoma, as is done with tissue, then these diagnostic phrases are insufficient in the newly diagnosed patient.

An incomplete list of articles regarding FNA diagnosis/classification of non-Hodgkin lymphoma just in the last 2 years is below.

FNA Diagnosis of Malignant Lymphoma 2000-2001

Reference	#cases	Sensitivity	Specificity	Subclass.
Di Cy 2001; 24:90 (Small Cell ML)	56	85%	100%	82%
AJCP 2000; 114:18	81	90%	100%	not done
AJCP 2000; 113:688	206	82%	85%	73%
Di Cy 2001; 24:1	127	88%	100%	77%
Cancer 2001;93:151(PTCL)	33	92%	100%	75%
Mod Path 2001; 14:472	139	95%	100%	77%
Total	**642**	**89%(x)**	**98%(x)**	**77%(x)**

Note that there is very high mean (>85%) for sensitivity and specificity in the ability to diagnose malignant lymphoma, and an average of almost 80% in the ability to subclassify these lymphomas according to the WHO classification when immunophenotyping is applied to these aspirates.

WHO Classification of Tumors of Lymphoid Tissues, B-Cell Neoplasms

- Precursor B-cell neoplasm
  - **Precursor B lymphoblastic leukemia/lymphoma.**
- Mature B-cell neoplasms.
  - **Chronic lymphocytic leukemia/small lymphocytic lymphoma**
  - B-cell prolymphocytic leukemia
  - Lymphoplasmacytic lymphoma
  - Splenic marginal zone lymphoma
  - **Mantle cell lymphoma**
  - **Follicular lymphoma**
  - **Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue(MALT-lymphoma)**
  - Nodal marginal zone lymphoma
  - Hairy cell leukemia
  - **Diffuse large B-cell lymphoma**
  - Mediastinal(thymic) large B-cell lymphoma
  - Intravascular large B-cell lymphoma
  - Primary effusion lymphoma
  - **Burkitt lymphoma / leukemia**
  - Solitary plasmacytoma of bone
  - Extraosseous plasmacytoma
  - **Plasma cell myeloma**
- B-cell proliferations of uncertain malignant potential
  - Lymphomatoid granulomatosis
  - Post-transplant lymphoproliferative disorder, polymorphic

Diagnoses in **Bold** are the more common forms of B-cell neoplasia.

Cytologic Features of Some of the More Common Forms of NHL

A. Mantle cell lymphoma(**MtCL**)

Clinical:

♣ biologically aggressive small cell B-cell lymphoma; about 6% of MLs
♣ occurs primarily in adults >50 years of age; rare in children
♣ affects men much more often than women(5:1)
♣ commonly extranodal as well as within lymph nodes
♣ most have disseminated disease(bone marrow) at the time of diagnosis
♣ much poorer prognosis than other small cell lymphomas with a median survival of only 2-5 years

Aspirates of MtCL:

∇ composition: monotonous small lymphocytes(slightly larger or similar in size to mature lymphocytes) in a single cell pattern
∇ nuclear irregularity/ grooves much less exaggerated than those seen in follicular lymphoma
∇ plasmacytoid lymphocytes, centroblasts, and paraimmunoblasts almost always absent
∇ occasional FDCs
∇ constant monomorphism with a complete lack of lymphocyte heterogeneity readily removes RLH from consideration.

Pitfall: An exception to the "rule" of lymphocyte monotony = lymphoma is that a minimally reactive lymph node can show little if any polymorphism, and thus imitate a small cell lymphoma. Clinical correlation is mandatory in every situation.

B. Small lymphocytic lymphoma(**SLL**)

Clinical:

∞ comprises about 6% of non-Hodgkin lymphomas
∞ primarily a lymphoma of older adults with patients under 40 being rare
∞ a low grade indolent lymphoma, but not amenable to therapy(**Table 12**)
∞ disease often widespread at the time of diagnosis [splenomegaly, hepatomegaly, peripheral blood, and bone marrow involvement common]
∞ asymptomatic patients often receive no treatment

Aspirates of SLL

♥ cellular uniformity with very high nuclear/cytoplasmic(N/C) ratios
♥cells minimally larger than mature lymphocytes
♥ nuclei in tissue sections are typically described as having coarse clumped chromatin – so-called clotted chromatin(cellules grumelées). In smears this coarse "block" type of chromatin pattern(best seen in Papanicolaou stained smears) may be repeated, or nuclei may have a dense smudged appearance
♥ nuclear borders are smooth or minimally irregular in SLL
♥ because of the presence of pseudofollicular growth centers, occasional transformed lymphocytes[prolymphocytes and paraimmunoblasts] are seen
♥ prolymphocytes: large lymphocytes with distinct nucleolus, modest amount pale cytoplasm
♥ paraimmunoblasts: slightly larger than prolymphocytes with perhaps a slightly larger nucleolus and more basophilic cytoplasm

These two types of transformed lymphocytes may not be distinguishable from each other in smears because of their overlapping features, nor is it necessarily critical to do so.

C. Follicular lymphoma(**FL**)

Clinical:

• vies with diffuse large B-cell lymphoma(DLBL) as the two most common forms of adult ML. From the NHL classification project, FL comprised 22% and DLBL 31% of non-Hodgkin lymphomas respectively[Blood 1997;89:3909-18]

• most patients older than 50 yrs.

- clinical presentation often asymptomatic, but vast majority(>80%) have disseminated disease[spleen, lymph node, & bone marrow involvement] at the time of diagnosis
- FL characterized by t(14:18) with rearrangement of the *bcl-2* gene in over 75% of cases
- antibody staining with *bcl-2* extremely helpful in distinguishing FL from follicular hyperplasia in tissue, but ineffective in smears because spatial relationship of staining cannot be evaluated
- current therapy of FL not curable

Aspirates of FL:

♥ rather than strict cellular monotony, smears of FL are much more apt to show some variation in cell size and nuclear outline

♥composed of a monotonous small lymphocytes, or more often, a mixture of transformed cells - centrocytes(small and large cleaved lymphocytes) and centroblasts(large non-cleaved lymphocytes) and small round lymphocytes

♥ nuclear notches/clefts and unevenness common; some appear to bisect or trisect a nucleus, while in others nuclear folds are more subtle

♥ FC fragments, TBMs rare, but dendritic cells present

♥ several variants. signet ring variant: conspicuous single cytoplasmic macrovacuole/stains with IgG/optically clear unlike the vacuoles of metastatic signet ring cell carcinoma that contain mucin. Signet ring morphology not unique to FL; has been described in SLL, DLBL, peripheral T-cell ML, and ALCL.

Summary of FNA Cytomorphology of Small Cell B-Cell Lymphomas

Lymphoma type	*Small Cell Morphology*	*Transformed Cells*
SLL/CLL	rounded	prolymphocytes/PBL
LpL	rounded, plasmacytoid, plasma cell	centroblasts, immunoblasts
Mantle Cell	rounded, slightly irregular	none
Follicular	cleaved, angulated, rounded	centroblasts, centrocytes
Marginal Zone	polymorphous: rounded, cleaved, monocytoid, plasmacytoid, plasma cell	centroblasts, centrocytes, immunoblasts

Clinical Features of Small Cell B-Cell Lymphomas

Type	Age/Gender	Stage I	Extranodal	Clinical Course
SLL/CLL	60/ m>f	none	rare	Indolent, incurable
LpL	60/ m>f	none	rare	Indolent, incurable
Mantle cell	60/ m>>f	rare	many	aggressive, incurable
Follicular	50/ m=f	rare	rare	indolent, rarely curable
Marginal zone/MALT	50/ m<f	often	usually	indolent, curable

Differential Immunophenotyping of Small Cell B-Cell Lymphomas

CD#	SLL	LpL	MtCL	FL	MZL	LL	Burkitt
CD5	pos	neg/pos	pos	neg	neg	pos/neg	neg
CD10	neg	neg	neg/pos	pos/neg	neg	neg/pos	pos
CD20	pos/neg	pos	pos	pos	pos	neg/pos	pos
CD23	pos	neg	neg	neg	neg	neg	neg
FMC-7	neg		pos	pos	pos		
CD43	pos	neg/pos	pos	neg	neg/pos	pos	neg
TdT	neg	neg	neg	neg	neg	pos	neg

SLL: small lymphocytic lymphoma; LP: lymphoplasmacytic lymphoma; MtCL: mantle cell lymphoma; FL: follicular lymphoma; MZL: marginal zone lymphoma; LL: lymphoblastic lymphoma.

D. Diffuse Large B-cell lymphoma(**DLBL**)

Clinical:

- comprises about 35% of all forms of adult lymphoma
- aggressive, but potentially curable
- affects a wide age range of adults, and is a major form of pediatric lymphoma
- about 40% present with extranodal disease
- primary mediastinal variant partial to young women, some have SVC syndrome

Aspirates of DLBL:

♣ lymphocytes are generally 3x or > small lymphocyte
♣ monomorphous large cells, or mixture with a minor percentage of small lymphocytes
♣ nuclei generally larger than those of tissue macrophages; round to irregular or multilobated; may be centroblastic, immunoblastic, or .bizarrely shaped
♣ nucleoli usually conspicuous; may be multiple or single
♣ cytoplasm is moderate to abundant; may include small vacuoles
♣ tingible body macrophages and necrosis common

E. Peripheral T-cell Lymphoma(**PTCL**), unspecified

Clinical:

- a heterogeneous category with a wide age spectrum
- accounts for about 6% of lymphomas
- nodal and extranodal[skin, subcutis, viscera] at the time of clinical presentation

Aspirates of PTCL, Unspecified:

∞ variable morphology; most common picture is large cell lymphoma, but also may have a mixture of small/intermediate lymphocytes and a high percentage of large lymphocytes
∞ large cells may simulate R-S cells
∞ variable number of eosinophils, plasma cells, and epithelioid histiocytes

Immunophenotyping of PTCL:

- absence of B-cell markers; **variable pan T-cell marker(CD1a, CD2, CD3, CD4, CD5, CD7, CD8) positivity usually with loss of 1 or more of these markers**, typically CD7. Cellular heterogeneity of PTCL can be confused with RLH. FC fragments and TBMs are uncommon to rare in PTCL, and their absence could be a clue that one is not dealing with a normal population of lymphocytes. Genotype shows a rearrangement of T-cell receptors.

F. Lymphoblastic Lymphoma(**LL**)

Clinical:

- an aggressive lymphoma comprising 30%-50% of pediatric lymphoma; M>F
- lymphadenopathy above the diaphragm; anterior mediastinal mass in up to 80% of patients
- may have clinical symptoms of bronchial asthma tracheal compression, or superior vena cava syndrome

FNA is an excellent initial step to establish diagnosis of LL. Several series report near perfect accuracy for the FNA diagnosis of LL. Since these patients often have a palpable neck mass and are critically ill[many with some degree of respiratory compromise], the expediency of FNA with minimal invasiveness, reliable accuracy, ability to procure cells for ancillary studies, and no requirement for sedation or general anesthesia demands that it be one of the first(if not the first) procedure used to secure a diagnosis.

Aspirates of LL:

∞ monotonous lymphoblasts cover the slide in a dissociated cell pattern
∞ little cell variation exists although a dimorphic pattern may occur due of the presence of smaller more hyperchromatic blasts
∞ lymphoblasts about 2x diameter of mature lymphocytes; conform to L_1-L_2 morphology of FAB classification
∞ nuclei typically rounded but may display some nuclear irregularity(convolutions)

G. Burkitt Lymphoma (**BL**)

Clinical:

¶ endemic in equatorial Africa; arises in the head and neck of young children; associated with EBV infection
¶ sporadic form predominates in North America; occurs in older children/teenagers; M:F, 3:1.
¶ BL can occur in adults, but at a much lower incidence
¶ most patients with sporadic BL present with intra-abdominal tumors[cecum, ileum]
¶ reported FNA sensitivity and specificity near 100%

Aspirates of BL:

- highly cellular; cells dispersed in a single cell pattern
- monotonously uniform cells 2-3 times diameter of mature lymphocytes
- nuclei rounded, coarse chromatin and 1-4 discrete nucleoli
- cell cytoplasm deeply basophilic, many coarse vacuoles(lipid filled)
- background LGBs also commonly vacuolated
- TBMs may be randomly dispersed throughout the smear mimicking the "starry sky" pattern
- because individual cell necrosis is common, a "dirty" background is typical.

	Lymphoblastic Lymphoma	**Burkitt Lymphoma**
Nucleoli	Inapparent, 1-2	Obvious, 1-5
Nuclear chromatin	Fine	Coarse, granular
Cytoplasmic vacuoles	Few, if any	Often numerous, obvious
Immunophenotype	Primarily T-cell, TdT+	B-cell, TdT negative

III. HODGKIN LYMPHOMA

The new WHO classification of HL has classified the tumor into two major subtypes:

WHO Classification: HODGKIN LYMPHOMA(Hodgkin's Disease)

- Classic Hodgkin Lymphoma(HL)
  - HL, nodular sclerosis, grade I and II
  - HL, lymphocyte-rich
  - HL, mixed cellularity
  - HL, lymphocytic depleted
- Nodular lymphocyte predominance HL (NLP HL)

The nodular LP form accounts for only 5-10% of cases while at least 50% are of the nodular sclerosis(N-S) subtype. Mediastinum often involved in N-S subtype.

The major limitation of FNA biopsy in HL is the inability to accurately separate NLP type from Classical type. Since these are 2 different disease entities and are treated differently then a tissue specimen must be obtained for a newly diagnosed patient.

A **Pitfall** in the FNA diagnosis of HL is the hypocellular smear secondary to densely fibrotic(sclerotic) node resulting in an inability to aspirate sufficient numbers of diagnostic R-S cells.

Clinical Features:

- painless enlargement most commonly of cervical or mediastinal lymph nodes
- common in North America. Bimodal age peak 2nd-4th decades of life; second smaller peak at 7th decade.
- Caucasians, HL comprises up to 35% of all lymphomas; only 5-10% in Orientals.
- either a single node or groups of nodes can be enlarged.
- "A" category without symptoms; "B" category with constitutional symptoms: include unexplained fever, drenching night sweats, and unexplained weight loss(>10%).
- no specific clinical laboratory abnormalities unique to HL
- modern chemo-/radiation therapy have led to a relatively high cure rate
- prognosis is not particularly dependent on histologic subtype, but on clinical stage, presence of absence of "B" symptoms, and patient age.

Aspirates of HL:

♠ presence of R-S cells, classic and variants, in a background of reactive hyperplasia

Mononuclear R-S cells:

♠enlarged(at least 20-30 μm) when compared to surrounding lymphocytes
♠may have bosselated nuclear contours
♠enlarged misshapen nucleoli; diameter = or > red cell
♠often apparent at medium power(due to ⇑ size
♠ R-S variants can "hide" in a sea of lymphocytes particularly when the smear is cellular
♠ Some cases only moderately enlarged bare R-S nuclei with no intact classic or mononuclear R-S cells. Stripped nuclei moderately enlarged with some lobulation of their nuclear contour. Because nucleoli in this form of R-S cell are smaller than classic R-S cells, and because the Romanowsky stain obscures rather than highlights nucleoli, this type of R-S cell can be overlooked.

Cytologic Mimics of Reed-Sternberg Cells

- Immunoblasts
- Megakaryocytes
- Dendritic Cells
- Large Cell Lymphoma(B and T-cell)
- Anaplastic Large Cell Lymphoma
- Melanoma
- Plasmablasts(Myeloma)
- Large Cell Carcinoma

Accuracy of FNA Diagnosis of Hodgkin's Lymphoma

Reference	# cases	Sens.	Spec.
Acta Cytol 2001; 45: 300.	170	84%	92%
Cancer 2001; 93: 52.	89	77%	100%
Mod Pathol 2000; 14: 472.	16	66%	100%
Cytopathology 1994; 5: 226.	62	97%	100%

Note that the ability to diagnose HL is high, but less than what is encountered in tissue sections. The major reason for false negative diagnosis is missing the classic and variant R-S cells. This can be secondary to their being overlooked because they are so few of them on the smears, or because the amount of fibrous tissue in the nodular sclerosis type of HL precludes extracting sufficient R-S cells into the thin FNA needle.

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QUESTIONS

1. Which of the following cannot be reliably diagnosed by Lymph Node FNA:
   - **A.** Small lymphocytic lymphoma.
   - **B.** Progressive transformation of germinal centers.
   - **C.** Diffuse large cell lymphoma.
   - **D.** Lymphoblastic lymphoma.
2. Immunoblasts can be found in smears of all these lesions except:
   - **A.** Mantle cell lymphoma.
   - **B.** Hodgkin's lymphoma.
   - **C.** Diffuse large cell lymphoma.
   - **D.** Marginal zone lymphoma

***Mark the appropriate letter.**

1. Quale delle seguenti entità non può essere diagnosticata tramite aspirazione del linfonodo con ago sottile:
   - **A.** Linfoma a piccoli linfociti.
   - **B.** Centri germinativi progressivamente trasformati.
   - **C.** Linfoma diffuso a grandi cellule.
   - **D.** Linfoma linfoblastico.
2. Gli immunoblasti si possono trovare nello striscio di tutte queste lesioni, tranne che in:
   - **A.** Linfoma della zona mantellare.
   - **B.** Linfoma di Hodgkin.
   - **C.** Linfoma diffuso a grandi cellule.
   - **D.** Linfoma della zona marginale.

***Contrassegnare la lettera giusta.**

ADVANCES IN THE PATHOLOGY OF CYTOMEGALOVIRUS INFECTIONS IN RENAL TRANSPLANT PATIENTS

Daniel D. Sedmak, M.D.
The Ohio State University
Medical Center
Columbus, Ohio

I. INTRODUCTION

Human cytomegalovirus (CMV) is a ubiquitous viral pathogen that is a cause of significant morbidity and mortality in neonatal and immunocompromised populations throughout the world. Cytomegalovirus (CMV), a betaherpesvirus, is the largest of the herpes viruses, measuring 150 to 200 nm in diameter. It contains a linear double-stranded DNA genome of 230 kb that encodes over 200 proteins. Viral replication occurs in a temporal fashion with sequential production of immediate early, early, and late proteins, many of which have been associated with host immune system interactions. Infection of cells is associated with cytomegaly, characterized by a large single intranuclear inclusion body and numerous basophilic cytoplasmic inclusions. As a betaherpesvirus, CMV has the ability to establish latency for the lifetime of the host. This ability is one of the cornerstones of CMV pathobiology: a significant percentage of CMV associated disease is the result of reactivation of latent virus.

The recorded history of human CMV extends back to the early 1900's when a clinical entity was reported by Jesionek and Kiolemeoglou that described the striking presence of nuclear and cytoplasmic inclusion bodies within cells of the lung, liver, kidney, and parotid glands of a leutic fetus (1). Identification of the etiologic agent remained elusive as reports accumulated on "Cytomegalic Inclusion Disease" (CID). Although Jesionek and Kiolemeoglou interpreted their findings as evidence for an infectious disease, most likely a protozoal organism, Cowdry suggested an alternative explanation in 1934 when he proposed that the "protozoan-like" cells in these patients were the result of a viral infection (2). It was not until 1956 that this virus was simultaneously isolated in tissue culture by Smith, Weller, and Rowe (3). Approximately 3 years later, Weller, Hanshaw, and Scott coined the name cytomegalovirus (4).

CMV infects up to 50-80% of all individuals by the sixth decade and transmission occurs by close person-to-person contact through oropharyngeal, cervical, and vaginal secretions, urine, spermatic fluids, breast milk, tears, feces, and blood (5). Acute infections typically are asymptomatic in healthy individuals but may result in a flu-like illness or a heterophile negative mononucleosis-like syndrome (5,6). These acute infections resolve into a state of quiescent latent infections lasting the lifetime of the host. Based upon serologic data and animal studies, it is probable that there are multiple episodes of subclinical and short-lived reactivation within latently infected individuals. In congenital or acquired immunodeficiencies of T lymphocyte-mediated immunity, acute infections and/or reactivation of persistent CMV often lead to significant clinical disease (5,6).

CMV targets numerous organs and tissues throughout the body and is associated with protean clinical syndromes. An analysis of the distribution of CMV antigens in autopsy material demonstrates the broad range of cell types infected during an acute infection: ubiquitously distributed epithelial cells, endothelial cells, and fibroblasts (7). Moreover,

peripheral blood leukocytes may be infected and aid in dissemination of the virus, and specialized parenchymal cells such as neurons in the brain and retina, smooth muscle cells of the GI tract, and hepatocytes may be infected (7).

CMV infections are serious and frequent sequelae of transplant immunosuppression regimens: 35 to 50% of bone marrow transplant recipients, 17 to 73% of heart and heart/lung recipients, and up to 92% of renal transplant recipients develop clinically significant CMV infections post-transplantation (8-12).

II. CYTOMEGALOVIRUS INFECTIONS IN RENAL ALLOGRAFT RECIPIENTS

Overview

One of the first reports of an association between CMV and renal transplantation was in 1964 when Hill et al. reported that CMV was a unique cause of pneumonia in renal allograft recipients (13). Since then, cytomegalovirus has been associated with a variety of extrarenal and intrarenal clinical disorders in renal transplant recipients (Table 1). The majority of these conditions result from *de novo* infection or reactivation of latent infection, with subsequent productive infection in a specific tissue or organ. Several, such as acute allograft rejection and acute allograft glomerulopathy, are more likely to represent indirect phenomena relating to selective immune system activation.

CMV and Acute Allograft Rejection

Simmons et al. first suggested a specific causal association between CMV infection and subsequent rejection in 1970 (14). In 1974, Lopez et al. published the first report of a specific association between CMV infection and the development of renal allograft rejection (15). Numerous studies have since supported this relationship (16-19); however, there is by no means universal acceptance of a cause and effect association (20-22). More recent support comes from therapeutic trials of CMV prophylaxis, which overall demonstrate a marked decrease in CMV disease and a parallel reduction in rejection episodes in those patients receiving pre-emptive therapy as compared to controls (23,24).

Animal models have further confirmed the hypothesis that CMV can induce and enhance rejection. In rat models of renal allograft rejection, CMV infection increased the number of acute rejection episodes (25) and the intensity of early graft inflammation (26).

There are multiple underlying considerations in the reported temporal relationship between CMV infection and rejection. First and foremost, there is universal agreement that immunosuppressive treatment of acute rejection results in a subsequent increase in CMV infection. Furthermore, the temporal CMV-rejection association may represent an increase in CMV replication in response to the cytokine milieu of rejection (27). Studies have specifically addressed these issues through analysis of the temporal relationship of infection to rejection (19) and through antiviral treatment of acute infection with resolution of rejection (18). They confirm that a subset of CMV infections is uniquely associated with subsequent rejection.

The basis for CMV induction of rejection may relate to its ability to induce alloreactivity. In the mouse, viral infections, including CMV, generate a marked increase in alloreactive cytolytic T lymphocytes (CTL) as well as virus-specific cellular immune responses (28,29). Critical experiments designed to test the hypothesis that CMV-induced alloreactive cells actually induce or accelerate rejection have yet to be performed.

The basis for CMV-enhanced alloreactivity is poorly understood, although there is no shortage of proposed mechanisms. These include CMV sequence homology and immunologic crossreactivity with the HLA DR beta chain (30), evidence for common antigenic determinants between CMV and the T cell receptor (31), enhancement of HLA class I antigen expression by type I interferons produced by infected cells (32), increased expression of ICAM-1 on infected cells (33, 34), and increased cytokine and chemokine release from infected cells (TNFα, IL-1β, IL-6, RANTES) (35-38). In addition, it has been shown that CMV-infected endothelial cells have the unique ability to activate CD4+-T lymphocytes isolated from seropositive individuals (39). In vitro, these activated CD4+ T cells are capable of inducing HLA class II and adhesion molecules on allogeneic uninfected endothelial cells (40,41).

CMV may also contribute to rejection through the direct and indirect activation of macrophages, which are important effector cells in both acute and chronic rejection (42-44).

CMV and Transplant-Associated Arteriopathy

The current leading cause of renal and cardiac allograft loss is chronic allograft damage, previously termed chronic allograft rejection. One of the most prominent manifestations of this chronic damage is transplant-associated arteriopathy (TXAA). This lesion differs from classic atherosclerosis in that it is characterized by concentric fibrointimal expansion of arteries by smooth muscle cells, fibroblasts and extracellular matrix, particularly collagen. In contrast to classic atherosclerosis, it often occurs along the length of a vessel. Many of the lesions contain foam cells and in some cases may develop into classic plaques, particularly in the coronary arteries of transplant patients with hyperlipidemia. The lesions result in marked narrowing of vessel lumens with down stream ischemic changes.

CMV was first associated with TXAA by Grattan et al. in 1989 (19). They noted an 18.5% higher incidence of severe coronary artery obstruction in CMV-infected cardiac allograft recipients as compared to CMV-negative recipients. Similar findings have since been reported by others (45-51). Most studies are focused on the association of CMV with cardiac TXAA, but the near identical pathology and time course of the lesions in kidney, pancreas, and some liver transplants has led to a general belief that CMV may be associated with the acceleration of these lesions as well.

Few studies have addressed the relationship between CMV and TXAA in renal allografts (52, 53). Almond et al. (52) reported that infections, including CMV, were a risk factor for chronic rejection histology in renal allografts. However, Nadasdy et al. (53) found no evidence of CMV DNA or antigen in renal TXAA lesions, suggesting that the association is not the result of direct infection of the vasculature. CMV infection has been shown to accelerate TXAA in rat renal allografts (26).

The mechanisms by which CMV accelerates TXAA development can be divided into those that damage the vasculature and those that contribute to the process of abnormal vessel wall repair. CMV may lead to vascular damage by enhancing alloreactivity, as previously reviewed. The pathologic consequence of pronounced alloreactivity is acute vascular rejection, a known precursor lesion of TXAA (54-58). CMV infection has been associated with acute vascular rejection in cardiac allografts (59); however, an association of CMV with acute cellular vascular rejection in renal allografts has not been reported.

There is minimal evidence to suggest that CMV directly damages allograft vessels. That is, it has been consistently demonstrated that CMV antigens and DNA are rarely present in cardiac and renal allograft vessels showing acute vascular rejection or TXAA (53,60-62). When found, the virus can be present in virtually all cell types, including endothelial cells, infiltrating

leukocytes and parenchymal cells (i.e., myocytes) (60,63-66). Interestingly, PCR studies for CMV DNA have reported greater percentages of CMV positive cases than ISH or IHC (67). This most probably reflects the increased sample size required by this technique, and hence the greater chance of detecting infiltrating CMV-positive leukocytes.

As previously indicated, CMV may abnormally accentuate the repair that follows allograft vasculature damage. Growth factor-induced smooth muscle cell proliferation and migration, and deposition of extracellular matrix characterize this repair phase. Interestingly, transcription of the growth factor TGFβ1 is increased in CMV-infected cells (68). Growth factor synthesis may be induced by cytokines released by CMV-infected cells. In this regard, increased expression of TNFα and IL-1 gene expression is seen in transfected and CMV-infected monocyte cell lines (35,36,69,70).

In addition to growth factors, CMV may be associated with TXAA through direct interaction of viral proteins with cell cycle-associated factors. Speir et al. (71) demonstrated that CMV protein IE84 binds to the cell cycle suppressor protein p53 and inhibits its activity. They suggested that binding of CMV IE84 to p53 within vessel walls results in aberrant smooth muscle proliferation.

Given the relative paucity of CMV antigens and DNA within allograft vessel walls and the low frequency of circulating infected leukocytes it seems most probable that CMV enhancement of TXAA relates to CMV-induction of alloreactivity and to CMV activation of macrophages. CMV-induced alloreactive lymphocytes, which are relatively frequent as compared to infected leukocytes or infected vessel wall cells, may release cytokines that induce growth factor synthesis by allograft vessel cells. In support of this hypothesis, Wagner et al. (72) found that alloreactive lymphocytes stimulate allogeneic endothelial cells to produce β FGF, TGF α and β, and PDGF A and B chains. In addition, type I interferons produced by CMV-infected cells, and interferon-gamma produced by activated natural killer cells and CMV-activated T lymphocytes, are potent inducers of macrophage activation. As previously indicated, macrophages are critical effector cells in rejection and TXAA (42-44), and inhibition of their function prevents the development of chronic rejection in rat renal allografts (73).

In summary, there is extensive epidemiological evidence for a role of CMV in inducing and enhancing acute allograft rejection in kidney. CMV-generated alloreactivity, and the nonspecific inflammatory component of macrophage activation, are the most probable mechanisms underlying these associations. The literature suggests that a definite cause and effect relationship between CMV and rejection only exists in a subset of CMV-infected transplant recipients, and that development of the association is dependent upon a host of other factors including the source and intensity of infection, the degree of histocompatibility, the genetic profile of the inflammatory response in the host, and extent of ischemic damage at the time of transplantation.

III. ACUTE ALLOGRAFT GLOMERULOPATHY AND CMV

Glomerular changes specific for renal allografts were first described over 35 years ago. These have been known by a variety of names including rejection transplant glomerulopathy, transplant glomerulopathy, endocapillary glomerulitis, glomerular transplant rejection, transplant glomerulopathy, allograft glomerulopathy, acute allograft glomerulitis, and acute allograft glomerulopathy (AAG) (74,75,76). These disorders can be summarized into two

basic glomerular processes: those that are more acute, i.e., AAG, and those that are chronic, i.e., transplant glomerulopathy.

The incidence of AAG ranges from 2.3% to 13.5% and has been reported as early as 2 days post-transplant and up to 180 post-transplant. The clinical manifestations of AAG generally include elevated creatinine, and proteinuria averaging 2+, although some patients have minimal renal failure and no proteinuria. Most patients have stable blood pressures and platelet counts.

The histopathology of AAG is characterized by focal to diffuse, and segmental to global, endocapillary proliferation with prominent endothelial cell swelling, intracapillary webs of PAS positive material, and scattered intracapillary mononuclear cells (76). Neutrophils are infrequently seen in AAG. In the Banff 97 working classification of renal allograft pathology, endothelial cell enlargement and an endocapillary mononuclear cell infiltrate define AAG (referred to as "early allograft glomerulitis"). Its grading is based upon the percentage of glomeruli involved and the extent of segmental or global involvement. In a study by Olsen et al. of 444 consecutive renal allograft biopsies, glomerulitis showed a strong pattern of clustering: if one biopsy from a patient had glomerulitis then it was highly probable that all biopsies from the patient would show some degree of glomerulitis (77).

In 1981, Richardson et al. reported an association between CMV viremia and AAG (78). Subsequent studies have not supported a clear relationship (74,79-81); however, the probable explanation for these diverse findings is that only a small subset of AAG presentations is secondary to acute CMV infection. Studies have rarely demonstrated evidence of CMV infection of glomerular cells during CMV-associated AAG episodes. If there is evidence of intrarenal infection, most positive cells are found within peritubular capillaries and represent either circulating leukocytes or infected endothelial cells (personal observation). Colvin et al have suggested that CMV promotes AAG development through induction of type I interferon synthesis and release (82). This interferon class has been shown to cause a selective increase in MHC class I molecules on glomerular and arterial endothelial cells. These molecules are targets for host cell anti-donor CD8+ T cells and antidonor antibodies.

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Table 1. Cytomegalovirus-Associated Disorders in Renal Allograft Patients

CMV viral syndrome (fever, leukopenia, thrombocytopenia, hepatitis) (5,6,8,83)
Acute cellular rejection (15)
Chronic allograft nephropathy (84)
Acute allograft glomerulopathy (78)
CMV hepatitis (5,6,7,8)
CMV pneumonia (5,6,7,8)
CMV ileocolitis (85,86)
CMV colitis with toxic megacolon (86)
CMV pancreatitis (5,6,7,8)
CMV menigoencephalitis (5,6,7,8)
Nephrogenic adenoma of ureter with CMV (87)
Recurrent Type I membranoproliferative glomerulonephritis (88)
Post-transplant thrombotic microangiopathies (89,90)
Necrotizing and crescentic glomerulonephritis (91)
Immunotactoid glomerulopathy (92)
CMV maculopapular rash (93)
Bowel infarction secondary to CMV-associated thrombosis (94)

QUESTIONS

1. Which of the following disorders have been associated with cytomegalovirus infection in renal allograft recipients?
   - **A.** Acute allograft glomerulopathy.
   - **B.** Recurrent membranoproliferative glomerulonephritis, type I.
   - **C.** toxic megacolon.
   - **D.** A and C.
   - **E.** All of the above.
2. Which of the following allograft kidney cell types has been shown to be infected with cytomegalovirus?
   - **A.** Endothelial cells.
   - **B.** Parietal epithelial cells.

C. Circulating leukocytes.
D. A and C.
E. All of the above.

***Mark the appropriate letter.**

1. Quale dei seguenti disordini è stato trovato associato con infezione da citomegalovirus in trapiantati renali?
   A. Glomerulopatia acuta da trapianto.
   B. Glomerolonefrite membrano-proliferativa ricorrente, tipo I.
   C. Megacolon tossico.
   D. A & C.
   E. Tutti quelli di sopra.
2. Quali dei seguenti tipi cellulari del rene trapiantato sono stati trovati infettati dal citomegalovirus?
   A. Cellule endoteliali.
   B. Cellule epiteliali parietali.
   C. Leucociti circolanti.
   D. A & C.
   E. Tutti quelli di sopra.

***Contrassegnare la lettera giusta.**

THE ROLE OF ULTRASTRUCTURAL EXAMINATION IN FIBROBLASTIC / MYOFIBROBLASTIC LESIONS

Brian Eyden PhD
Head of Diagnostic Electron Microscopy
Department of Histopathology
Christie Hospital National Health Service Trust
Manchester M20 4BX
United Kingdom
Tel +44 161 446 3292
Fax +44 161 446 3300
Email brian.eyden@christie-tr.nwest.nhs.uk

INTRODUCTION

Most connective tissue cells, such as the fibroblast, smooth-muscle cell, pericyte, adipocyte, have clearly defined tumoral equivalents which have been recognised for some time. The myofibroblast, however, has been identified only comparatively recently (Gabbiani et al 1971; Ryan et al 1974), and for a number of reasons, it is a less well understood cell. In spite of this, many lesions have been classified as myofibroblastic. For some of these lesions (especially nodular fasciitis, the fibromatoses – including the nodular palmar variant, Dupuytren's disease – and inflammatory myofibroblastic tumour) there is widespread agreement on their myofibroblastic nature. For other lesions, some authors have found the evidence for myofibroblastic differentiation unconvincing (for example, the myofibroblastomas – Eyden et al 1996, 1999; Eyden and Chorneyko 2001). Such controversies have arisen partly because of the difficulty in reaching an agreed definition for the myofibroblast. Because the myofibroblast was initially defined in ultrastructural terms (Gabbiani et al 1971; Ryan et al 1974), electron microscopy has been central to identifying the myofibroblast and diagnosing myofibroblastic tumours and lesions. More recently, the convenience of a definition based on light microscopy and immunohistochemistry has been emphasised (Mentzel et al 1998; Bisceglia and Magro 1999). Within this context, this presentation deals with the main applications and controversies of electron microscopy in diagnosing fibroblastic/myofibroblastic lesions.

DEFINING THE MYOFIBROBLAST

Diagnosing tumours partly consists of identifying features observed in a putative normal cellular counterpart: for example, S100 protein and melanosomes in malignant melanoma, and α-sarcomeric actin and sarcomeres in rhabdomyosarcoma. For myofibroblastic lesions, however, there is, strictly speaking, no "normal" cellular counterpart: with one or two exceptions (Garant 1976; Holstein et al 1996), the myofibroblast is not found in quiescent untraumatised adult tissues (cf Yang et al 1996; Hisaoka et al 1998; Mentzel et al 1998). Myofibroblasts in granulation tissue and tumour stroma are argued here as the nearest equivalent to the normal cell counterpart with which to define and identify myofibroblastic lesions. Such myofibroblasts (referred to here for convenience as "normal" myofibroblasts) are recognised by characteristic light and electron microscope features. They are spindled cells with fusiform nuclei, set in a more or less collagenised matrix, with a rather ill-defined cytoplasm, which is paler and less fibrillar than the usually brightly eosinophilic cytoplasm typical of smooth-muscle cells (Mentzel 2001). They stain for α-smooth-muscle actin, fibronectin, and vimentin. Lesional myofibroblasts also express desmin staining, a finding which requires some comment. Tumours cannot be

expected to mirror exactly their putative normal cell counterparts (Mentzel 2001): the genetic abnormalities which characterise the neoplastic process can explain loss of anticipated features, as well as the appearance of unexpected ones (so called *aberrant* or *anomalous* findings in the case of immunohistochemistry). Desmin staining in myofibroblastic lesions is interesting because granulation tissue and tumour stroma myofibroblasts express very little of this intermediate filament (Skalli et al 1989; Truong et al 1990), and desmin, therefore, should not be considered part of the *primary* definition of the myofibroblast, even though it is recognised in some lesions. It may be regarded as being *compatible* with myofibroblastic differentiation, but arguably it would be inappropriate to use it to provide strong *support* for a myofibroblastic diagnosis. By analogy, for example, the fair number of malignant melanomas which are positive for cytokeratin would not justify making cytokeratin a primary marker for melanoma like S100 protein or HMB45.

The ultrastructure of the "normal" myofibroblast is best be appreciated in terms of the concept of a reactive product of a stromal connective tissue cell. The widely accepted working hypothesis is of a quiescent fibroblast, which, on activation, synthesises abundant rough endoplasmic reticulum cisternae (rER) for matrix production, then switches on genes for smooth-muscle actin myofilaments for contractility (Welch et al 1990). These two ultrastructural features especially (but also, for example, gap junctions and collagen secretion granules – Eyden 1989; Eyden et al 1991; Eyden 1996; Schürch et al 1998) are widely understood and accepted as markers for the myofibroblast. This cell, however, also has a further distinctive feature, at the cell surface – the fibronexus - which is less well recognised and which is argued here as a significant marker for myofibroblastic differentiation.

The first detailed ultrastructural descriptions of the myofibroblast (Gabbiani et al 1971; Ryan et al 1974) not only described rER and myofilaments but also a component on the cell surface designated as basement membrane-like material. Further study showed that this differed from basal lamina ("basement membrane") and represented the external component of a cell-surface specialisation called the *fibronexus* or *fibronexus junction* (Ryan et al 1974; Singer et al 1984; Eyden 1993, 2001a). At the fibronexus, intracellular myofilaments and extracellular fibronectin filaments (these forming the fibronectin fibril - Eyden 1993, 2001a) converge to form a cell-to-matrix adhesive device. Fibronexus junctions have been promoted as an important myofibroblastic feature for two reasons: they formed part (albeit under a different terminology) of the original definition of the granulation tissue myofibroblast (Gabbiani et al 1971), and they are consistently and sometimes exuberantly expressed in tumour stroma myofibroblasts (Ryan et al 1974; Eyden 1993, 2001a,b).

These features – fibronexus junctions, prominent rER, and modestly developed and mainly peripheral myofilaments – in an appropriate histology and immunophenotype, constitute one basis for defining the myofibroblast and identifying myofibroblastic differentiation in tumours and tumour-like lesions. The features collectively amount to the highest level of myofibroblastic differentiation possible ("fully differentiated" myofibroblasts). The extent to which these and light microscopy features are found in a wide variety of lesions varies considerably.

SPECTRUM OF DIFFERENTIATION IN MYOFIBROBLASTIC LESIONS

Using the ultrastructural criteria of fibronexus junctions, prominent rER and modest myofilaments as representing the highest level of myofibroblastic differentiation (in a consonant histological and immunophenotypic picture), a spectrum of myofibroblastic differentiation is evident in tumours and tumour-like lesions. Those lesions containing the most highly differentiated myofibroblasts are hypertrophic scar, Dupuytren's disease and other fibromatoses, nodular and proliferative fasciitis, and inflammatory myofibroblastic tumour. Those lesions with lesser but still distinct myofibroblastic differentiation include keloid, fibroma of tendon sheath, post-operative spindle-cell tumour, and myofibrosarcoma. These more poorly differentiated lesions depend to a certain extent for their identification on the ability to identify fibronectin fibrils on their own, since the fibronexus-associated myofilament bundles may be absent or sparse. In this respect, it is important to be able to distinguish the fibronectin fibril of the myofibroblast from the lamina of smooth-muscle differentiation.

Compared with lamina, the fibronectin fibril tends to be more densely staining, straighter in profile, with a finely filamentous substructure, often a co-linear relationship with nearby intracellular myofilaments and is often seen diverging away from the cell surface. It is worth emphasising that the fibronexus and the fibronectin fibril are under-interpreted structures in that several instances exist where these structures are illustrated but either not mentioned or misinterpreted as lamina or basement-membrane-like material of indeterminate significance: examples include nodular and proliferative fasciitis (Wirman 1976; Craver and McDivit 1981), myositis ossificans (Povýšil & Matejovský 1979), as well as some fibromatoses (Viale et al 1988) and inflammatory myofibroblastic tumours (Chou et al 1988). In short, the incidence of the fibronexus or fibronectin-fibril is higher than is recognised, and true myofibroblastic differentiation is arguably more widespread than perceived.

Often, these myofibroblastic lesions are described as consisting of myofibroblastic *and* fibroblastic cells to reflect observations that not all cells have the ultrastructural features of myofibroblasts. However, some lesions, such as elastofibroma (Ramos et al 1978), appear to be fibroblastic, their designation as myofibroblastic being due to a misinterpretation of intermediate filaments as muscle filaments.

SMOOTH-MUSCLE DIFFERENTIATION IN SO CALLED MYOFIBROBLASTIC LESIONS

Just as some so called myofibroblastic lesions are really fibroblastic, there are compelling reasons on the grounds of ultrastructure and/or strong desmin staining for regarding certain other lesions as expressing a kind of smooth-muscle (non-myofibroblastic) differentiation. While perhaps most of the ultrastructurally examined fibromatoses, as already indicated, exhibit fibronexus junctions and so are fully myofibroblastic, a few others have lamina indicative of smooth-muscle cells (Takagi et al 1991) or are strongly desmin-positive (Hasagawa et al 1990), a feature suggesting smooth-muscle rather than or in addition to myofibroblastic differentiation. It is interesting that, according to Hasegawa's paper, Dupuytren's disease and nodular fasciitis, which are known for their well defined myofibroblastic credentials on the basis of fibronexus junctions (Tomasek et al 1987; Eyden 2001a), are far less desmin-reactive than extra-abdominal desmoid. Further, in separate studies of 103 examples of nodular fasciitis, none were reactive for desmin (Montgomery and Meis 1991, Thompson et al 2001).

In identifying smooth-muscle differentiation fine structurally, attachment plaques with overlying lamina (sometimes with intervening plasmalemmal caveolae) constitute an important smooth-muscle feature distinct from the fibronexus of the myofibroblast (Eyden 2001b). These surface features have been seen in a number of myofibroblastomas (Michal et al 1993; Tanda et al 1993; Eyden et al 1996, 1999; Eyden and Chorneyko 2001). By contrast, in only one study is there a suspicion of a fibronectin fibril (Padberg et al 1994). Many of the tumours referred to as myofibroblastomas may, therefore, be exhibiting a kind of (non-myofibroblastic) smooth-muscle differentiation. This argument, especially given the strong desmin staining in some cases, applies also to the angiomyofibroblastomas. Here, the cytoplasm contains little of the rER expected of the myofibroblast, no myofilaments, the fibronexus has never been identified, and one study has shown smooth-muscle lamina (Fletcher et al 1992).

THE LIGHT MICROSCOPY DEFINITION OF THE MYOFIBROBLAST

Other so called myofibroblastic lesions which are arguably showing a form of smooth-muscle differentiation include paratesticular plexiform tumor (Busmanis 1991), leiomyomatosis peritonealis disseminata (Pieslor et al 1979) (which appears to be expressing a synthetic or matrigenic smooth-muscle phenotype to judge by the prominent rER), and massive ovarian edema (Roth et al 1979). In certain other lesions – dermatofibroma (Zelger et al 1997), Dermatofibrosarcoma protuberans (Ma et al 1992; Dominguez-Malagon et al 1995), atypical fibroxanthoma (Ma et al 1992), dermatomyofibroma (Colome and Sanchez 1994), pseudoangiomatous stromal hyperplasia (Powell et al 1995) - the evidence for myofibroblastic differentiation is based either on the less strict ultrastructural combination of rER with myofilaments (without fibronexuses), or the light microscopy definition encompassing spindle-cell morphology and immunstaining for α-smooth-muscle actin.

This light microscopy definition merits attention because of the acknowledged decline in the use of electron microscopy in routine tumour diagnosis (Mentzel et al 1998; Bisceglia and Magro 1999). It remains an argument held by some authorities, however, that the light microscopy definition is more imprecise and open to greater interpretational uncertainty in the sense that a variety of spindled cells can express α-smooth-muscle actin – smooth-muscle cells, myofibroblasts, pericytes, myoepithelial cells, epithelium undergoing mesenchymal transformation (Eyden 2001b). To illustrate this point further, electron microscopy can distinguish, for example, a myofibroblast from a matrigenic smooth-muscle cell, i.e., one which has transdifferentiated from the contractile to the synthetic phenotype, and which, in spite of containing abundant rER and relatively few peripheral myofilaments like a myofibroblast, differs from it by its smooth-muscle cell surface (Eyden 2001b). The ultrastructural definition is useful, therefore, in situations of diagnostic uncertainty associated with spindle-cell tumours positive for smooth-muscle actin and or desmin. An additional area of utility is for intra-abdominal spindle-cell lesions positive for c-kit, some of which are true gastro-intestinal stromal tumours while some are true myofibroblastic lesions (Yantis et al 2000).

MYOFIBROBLASTIC MALIGNANCIES

The early studies on tumour stroma and granulation tissue emphasised the essentially reactive nature of the myofibroblast. Increasingly in recent years, however, spindle-cell malignancies with myofibroblastic differentiation have been documented (Vasudev & Harris 1978; Eyden et al 1991, 1992; Taccagni et al 1997; Montgomery et al 2001; Bisceglia et al 2001). Some of these have presented as low-grade spindle-cell sarcomas

which before analysis by electron microscopy had been diagnosed as leiomyosarcoma because of smooth-muscle actin immunostaining (Eyden et al 1992): here, fibronexuses and strong fibronectin immunostaining confirmed myofibroblastic differentiation. Other myofibrosarcomas have showed an aggressive lethal course (Taccagni et al 1997). With certain relatively poorly differentiated sarcomas for which a diagnosis of myofibrosarcoma may be considered, cell surface material may be encountered ultrastructurally which cannot unambiguously be identified as lamina or fibronectin (Bisceglia et al 2001). Consequently, it may be necessary to construct a "most likely" diagnosis of myofibrosarcoma, based on combining both light and electron microscopy features.

The complex and controversial entity, malignant fibrous histiocytoma, also contains variable numbers of myofibroblasts as neoplastic elements (Meek 1984; Montgomery & Fisher 2001).

TABLE

Keloid	
Santucci et al 2001	SMA+, FN+, rER+, MF+, FNX+
Hypertrophic scar	
Santucci et al 2001	SMA+, FN+, rER+, MF+, FNX+
Nodular fasciitis	
Wirman 1976	rER+, MF+, FF+
Eyden 2001a	FNX+
Proliferative fasciitis	
Craver and McDivitt 1981	rER+, MF+, FF+
Proliferative myositis	
El-Jabbour et al 1991	SMA+, rER+, MF+, DES+
Myositis ossificans	
Povýšil and Matejovský 1979	rER+, MF+, FF+
Fibromatosis (myofibromatosis)	
Viale et al 1988	MSA+, SMA+, rER+, MF+, IB+, FNX
Takagi et al 1991	rER+, MF+, L+
Tomasek et al 1987	rER+, MF+, FNX+
Inflammatory myofibroblastic tumor	
Chou et al 1988	actin+, DES+, rER+, FF+
Eyden 2001a	FNX+
Post-operative spindle cell nodule	
Papadimitriou/Drachenberg 1994	SMA+, DES+, CK+, rER+, MF+, ?FF+
Fibroma of tendon sheath	
Eyden 2001a	FF+

Myofibrosarcoma

Vasudev and Harris 1978	rER+, MF+, FNX+
Eyden et al 1991	SMA+, rER+, MF+, FF+
Eyden et al 1992	SMA+, FN+, DES-, rER+, MF+, FNX+
Taccagni et al 1997	SMA+, FN+, rER+, MF+, FNX+
Montgomery et al 2001	SMA+, DES+, rER+, MF+, FNX-
Mentzel et al 1998	SMA+, DES+, FN+, rER+, MF+, L+
Bisceglia and Magro 1999	SMA+, DES-
Bisceglia et al 2001	SMA+, rER+, MF+, ??FF??L

Malignant fibrous histiocytoma

Fukuda et al 1988	rER+, MF+, FF+
Meek 1994	CK+, MF+, FF+

Myofibroblastoma

Suster and Rosai 1989	muscle actin+, DES-, rER+, MF+
Weiss et al 1989	actin+, DES+
Michal et al 1993	rER+, MF+, AP+, L+
Tanda et al 1993	SMA+, DES-, rER+, MF+, AP+, L+
Padberg ert al 1994	actin+, rER+, MF+, FF+?
Eyden et al 1996	SMA+, DES-, rER+, MF+, L+
Eyden et al 1999	SMA+, DES+, rER+, MF+, L+

Angiomyofibroblastoma

Fletcher et al 1992	DES+, SMA-, rER+, IF+, MF-, L+

Superficial cervico-vaginal myofibroblastoma

Laskin et al 2001	DES+,SMA+, MSA+

Dermatofibroma

Zelger et al 1997	SMA+, rER+, IF+, AP+, focal L+

Dermatofibrosarcoma protuberans

Ma et al 1992	MSA+, DES-
Dominguez-Malagon et al 1995	SMA+, rER+, MF+

Atypical fibroxanthoma

Ma et al 1992	MSA+, DES-

Dermatomyofibroma

Colomé and Sánchez 1994	MSA+, rER+, MF+, L+

Plexiform tumour

Busmanis 1991	MSA+, DES+, MF+

Leiomyomatosis peritonealis disseminata

Pieslor et al 1979	rER+, MF+, AP+

Massive ovarian edema

Roth et al 1979	rER+, MF+, AP+, L+

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** This is abstracted from a review on myofibroblastic lesions to be submitted to *Seminars in Diagnostic Pathology* for 2002 **

QUESTIONS

(See the opposite page).

QUESTIONS

1. Name two lesions where significant numbers of lesional cells consist of fully differentiated myofibroblasts.
   Indicate the names: ....., ...
2. List the three principal ultrastructural features of the fully differentiated myofibroblast.
   Indicate the names: ....., ..., ...

1. Nomina due lesioni in cui un consistente numero di cellule lesionali sia rappresentato da miofibroblasti ben differenziati.
   Citare i nomi: ....., ...
2. Elenca i tre principali aspetti ultrastrutturali del miofibroblasto ben differenziato.
   Elencare i nomi: ....., ..., ...

SURGICAL PATHOLOGY –SESSION IV. KIDNEY & ADRENAL

CASE PRESENTATIONS (4 CASES)

Case 14. Mixed epithelial-stromal tumor of the kidney

(*M. Bisceglia*)

Case 15. Angiomyoadenomatous tumor of the kidney

(*M. Michal*)

Case 16. Oncocytic borderline tumor of the adrenal gland

(*M. Bisceglia*)

Case 17. Corticomedullary mixed tumor of the adrenal

(*M. Michal*)

CASE 14. 91557-93

M. Bisceglia, Servizio di Anatomia Patologica, IRCCS-Ospedale "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy

CLINICAL HISTORY

A 65-year old woman with a huge tumor that was incidentally discovered in the right side of the abdomen during investigation for cholelitiasis. The patient underwent cholecystectomy and right radical nephrectomy which included the tumoral mass.

PATHOLOGICAL FINDINGS.

GROSS FEATURES

The tumor was 14 cm in size and attached to the anterior surface of the kidney by a short and broad peduncle, having an almost total exophytic growth. The external surface of the tumor mass was mainly smooth or in places bosselated. On sectioning the cut surface revealed a biphasic appearance with a large multimacro-microcystic peripheral component and a solid -partly centrally located- area of "fibromyomatous" consistency with satellite nodules of about 8 cm in aggregate.

HISTOLOGICAL APPEARANCES

Multiple sections confirmed the anticipated biphasic gross aspects, showing the solid component having the appearance of a mesenchymal spindle cell neoplasm with several mitoses (in places up to 10 mitoses per 10HPF), focal hemorrhages and minute foci of necrosis. The variously sized cysts of 1-5 cm, more or less juxtaposed, were lined by a low cuboidal epithelium and separated by variuosly thickened stromal septa of hypercellular (ovarian-like) tissue which in places was more condensed in close vicinity to the cyst walls. The stromal septa displayed even occasional scattered embryonal-like, inactive, small tubular structures as an integrant part of the tumor itself. Immunohistochemically the "fibromyomatous" nodular solid component was widespread immunoreactive for vimentin and focally for alpha-smooth muscle actin and muscle-specific actin (HHF-35), whilst it was negative for desmin, S100-protein, HMB-45, and cytokeratins. The stromal septa of the multicystic portion of the tumor also expressed the same antigenic profile as seen in the wide central solid portion, while the epithelial-looking layers lining the cysts were as expected reactive for cytokeratins and negative for vimentin. The small tubules which were embedded in the stromal septa were even immunoreactive for cytokeratins.

SPECIAL STUDIES

An electron microscopic study of the solid fibromyomatous component only was performed which documented a fine structure of spindle cells with fibroblastic and early myofibroblastic differentiation.

A cytofluorimetric analysis of the same solid tumor component displayed a near-diploid DNA content with DNA Index 1.2 .

ORIGINAL DIAGNOSIS

In 1993 based on the above findings a diagnosis was signed out of a "combined tumor" with a solid (congenital) mesoblastic nephroma component -for which the designation of "atypical" was warranted by virtue of mitoses and foci of necrosis and hemorrhages- and a cystic nephroma component.

After the present diagnosis was made, at that time, the same case was sent for consultation to two referral centers, where it was diagnosed as a "cystic nephroma with sarcomatous overgrowth" and as "mesoblastic nephroma, with atypical and cystic features", respectively.

DISCUSSION

(Congenital) mesoblastic nephroma, also eponimically named "Bolande's tumor" after the patronimic author who first coined the term in 1967 (1) and subsequently separated it from Wilms' tumor, with which it was previously confused, is a rare pediatric tumor of kidney with the highest peak of incidence during the first 3 months postnatally and an "almost" constant favourable prognosis. Other synonyms of the tumor are: fetal mesenchymal hamartoma and leiomyomatous hamartoma.

In regard with the histogenesis of (congenital) mesoblastic nephroma the debate is still open. As a matter of fact there are on one side some authors who believe that the spindle cells which constitute the tumor derive from the secondary mesenchyme ("induced mesenchyme") which lacks the capacity to form epithelial structures, whilst on the other side other authors think that the tumor cells take origin from the primary mesenchyme ("mesoblast" or "metanephric blastema") which is aknowledged with that ability. Based on the latter theory –which is supported by reports of cases endowed of epithelial structures as an integrant component of the tumor itself- (congenital) mesoblastic nephroma has the same histogenesis of Wilms' tumor (i.e, from primary mesenchyme), in which the epithelial component usually progresses to a terminal differentiation, whereas the mesenchymal component undergoes the stromagenic committment typical of secondary mesenchyme.

Two histological subtypes are currently distinguished at histology: the conventional or leiomyomatous type and the atypical or cellular type, the former being characterized by interlacing bundles of spindle cells lacking a significant mitotic activity (0-8 mitoses per 10 HPF), while the latter exhibits a densely cellular proliferation, shorter spindle or oval cells, high mitotic index (8-30 mitoses per 10 HPF), cystic degeneration, necrosis and hemorrhage (2). Mixed forms are also on record with a combination of the two patterns. Recurrence and even death which are well on record in literature were always related to the atypical form or to the mixed form, expecially in patients aged more than 3 months and in those cases in which the surgical removal was not complete (3).

According to the literature this tumor is rare in late childhood (the peak age of the incidence of Wilms' tumor), and even exceptional in adulthood, with only 17 cases on record up to 1993 (4,5) and 15 further cases up to 2001 (5), which –in addition to 4 more cases published under the term of "cystic hamartoma of the renal pelvis" (the same as mesoblastic nephroma of adulthood apart from location [6])- make a grand total of 36 cases ever reported in adulthood under various synonymic terms. Likely even the case reported by Kural et al (7) as multilocular cystic nephroma (unusually involving the renal pelvis and so probably representing another example of the cystic hamartoma of the pelvis) should be included in the count of (congenital) mesoblastic nephromas of adulthood.

Congenital mesoblastic nephroma is usually solid, but cystic variants –mainly pertinent to the atypical variant- have also been described (3). The differential diagnosis includes true leiomyomatous tumors (leiomyoma –a term which was improperly also synonymically used to refer to congenital mesoblastic nephroma- and leiomyosarcoma), angiomyolipoma, nerve sheath tumors, clear cell sarcoma of the kidney, and nephrogenic adenofibroma.

Cystic nephroma is the suggested term in alternative and replacement of multilocular cyst and indicates the benign counterpart of Wilms' tumor. Definition of this entity has been well elucidated for years (8): while on one side it includes the overall tumor composition of cysts and septa, the epithelial lining of the cysts and the fibrous composition of the septa in which well differentiated tubules may also be present, on the other side it excludes any nodular solid tumor growth as well as the presence of blastemal cells or immature tissues. In infants cystic nephroma forms a continuous spectrum with cystic partially differentiated

nephroblastoma (8-9), another muticystic tumor resembling the former but containing immature elements (blastemal cells with or without embryonal stromal or epithelial elements) (8). It has also been recognized in adults with less than 45 cases on record in the world literature during the decade 1982-1993 (5,10,11,12). Cystic nephroma in adults exhibits septal stroma usually cellular (hypercellular variant) and often -at least in places- of the ovarian-like type (11).
Cystic nephroma (intended as a terminally differentiated (mature) variant of Wilms' tumor) and (congenital) mesoblastic nephroma are probably congeners in accordance with the unitary concept of the histogenetic theories on renal neoplasms: this assumption giving explanation both for the cohexistence of focal epithelial component in some mesoblastic nephromas.
In essence my opinion was that the case herein presented probably was a tumor from the primitive mesenchyme of the metanephric blastema, which in the cystic areas shows the mesenchymal and epithelial features of a cystic nephroma, while in others it differentiates towards the secondary mesenchyme with the result of the multinodular area of a (congenital) mesoblastic nephroma. In the literature there were only few cases alluding to the possibility of the existence of such cystic and solid combined tumors in adults (10,13,14,15).

In 1993 this case was also circulated among the members of this Club, with most of them agreeing with the proposed diagnosis (otherwise, out of 17 opinions expressed two were in favour of a mixed Mullerian tumor and two favoured a multilocular cystic nephroma with sarcomatous areas).

FINAL DIAGNOSIS

Recently, following the ultimate literature focusing on this peculiar biphasic, solid and cystic, tumor of kidney, which has been descriptively qualified as "mixed epithelial/stromal tumor of the kidney", the tumor herein presented has been reevaluated and as such nosologically categorized.

COMMENT AND CONCLUSIONS

"Mixed epithelial and stromal tumor of the kidney" (MESTK) seems to be a distinctive group-entity of tumors, which has been quite clearly delineated during the last 4-5 years. As far as the original patronimicity is concerned it was Michal and Syrucek (16) who -in a case report of such a tumor which was published in 1998- had the merit to coin a clear-cut descriptive definition for this neoplasm, a term which independently in the same year was also proposed by Eble and Bonsib for the cystic hamartoma of the renal pelvis (17). Subsequently Adsay et al popularized the acronym MESTK thanks to their seminal paper dealing with a large series of such tumors (18). MESTKs have been disclosed (19) to lack the specific genetic alterations (especially trisomy 11 and translocation t(12;15) (p13;q25) associated with ETV6-NTRK3 gene fusion) usually found in the cellular variant of congenital mesoblastic nephroma as well as in congenital fibrosarcoma of soft tissue.
Further in a letter to the editor concerning with benign mixed epithelial and stromal tumors of the kidney Michal (20) originally proposed the unifying view of embracing in the same group-entity all those tumors of adults (mostly perimenopausal women) characterized by a biphasic epithelial and stromal appearance, with the epithelial component possibly being cystic or adenomatous.
Thus –according to Michal (20) and Adsay (18)- adult mesoblastic nephroma, cystic nephroma with hypercellular (ovarian-like/Müllerian-like) stroma (also called multilocular cyst of the kidney) (11, 21), cystic hamartoma of the kidney, adult mature nephroblastoma

(22) are simply different architectural aspects in the spectrum of the same entity, which is classically benign: pathological implications previously heralded by some previous concepts suggesting that so-called mesoblastic nephroma in adults is completely different (6) from the congenital mesoblastic nephroma and that cystic nephroma of the adult should biologically as well as histogenetically be separated from the infantile counterpart (17).

MESTK patients usually present with flank pain and hematuria or may be even asymptomatic (28% of cases in Adsay's series [18]), with tumors incidentally discovered during investigations for other diseases. A female to male sex predominance of 6:1 with a mean age of 46 years, and a probable hormonal influence has also been documented. Immunophenotypically the spindle cells are positive for vimentin, muscle markers (desmin and muscle actins), and estrogen and progesterone receptors. The epithelial components of the tumor shows expression for cytokeratins and focally for vimentin. Spindle and epithelial cells are negative for S-100 protein, HMB-45, and CD34.

The differential diagnosis of a mixed epithelial stromal tumor include the following possibilities: the solitary fibrous tumor (entrapping tubular epithelial structures), the true leiomyoma/leiomyosarcoma, the angiomyolipoma (without adipocytic component), the sarcomatoid renal cell carcinoma, the extragastrointestinal GIST, the exceptional adult occurrence of a cystic partially differentiated nephroblastoma (23), and the primary synovial sarcoma of the kidney (24), with the last two as the main diagnostic challenges. However, the former should be recognized based on the presence of blastemal cells with or without immature mesenchymal or epithelial derivatives in the septa outlining the cysts (8-9); the latter by the specific molecular SYT-SSX fusion transcripts. A case of malignant transformation of the mesenchymal component in a classic mixed epithelial stromal tumor of the kidney has also been reported (25) with the sarcomatous changes morphologically identical to the monophasic fibrous synovial sarcoma of the kidney, with possible examples thereof probably in part previously described under different terms (11,26). Likely, mainly based on their typical age of occurrence in infancy and childhood, some recently recognized pediatric tumors of mixed type (i.e. nephrogenic adenofibroma [27], subsequently renamed as metanephric adenofibroma [28]) or pure stromal type (i.e. metanephric stromal tumor [29]), should not be difficult to differentiate from MESTK, even though a case of a mixed tumor of such type with malignant stromal component (30) has been also described in a young adult patient. Ultimately the socalled renal medullary fibroma (subsequently renamed as renomedullary interstitiall cell tumor) should be included in the differential (31).

In conclusion, here we have presented a case of MESTK, a newly delineated entity.

This case -which has been only recently reclassified as such- around ten years ago represented a prototypical example of a simultaneous combination of the two alternate diagnostic labellings (cystic nephroma and mesoblastic nephroma in combination) under which at that time these tumors used to be diagnosed.

Follow-up: 9 years after the surgical intervention the patient is still alive and free of disease.

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QUESTIONS

1. A. Is congenital mesoblastic nephroma the same entity as mesoblastic nephroma in adulthood? **Yes or No. ____.**
   B. Which the current popular name is for alluding to the mesoblastic nephroma in adulthood?
   Name It: .....
2. Is the cystic hamartoma of the renal pelvis the same as mesoblastic nephroma of adulthood in that location? **Yes or No**. ____.

1. A. Il nefroma mesoblastico congenito rappresenta la stessa entità del nefroma mesoblastico dell'adulto? **Si o No. ____.**
   B. Quale è il nome divulgato negli ultimi anni per fare riferimento al nefroma mesoblastico dell'adulto?
   Riportare il nome: .....
2. L'amartoma cistico della pelvi renale è la stessa entità del nefroma mesoblastico dell'adulto in quella particolare sede anatomica? **Si o No**. ____.

CASE 15. M30280/99

Michal Michal, M.D., Sikl's Department of Pathology, Charles University, Medical Faculty Hospital, Pilsen, Czech Republic.

CLINICAL HISTORY

A-93-year old Caucasian male was hospitalized with enterorrhagia, anemia and melena. Ultrasonographic examination and CT revealed a solid tumor of the left kidney. Intravenous pyelography confirmed the presence of a space-occupying lesion in the left kidney. The left kidney showed normal shape. Total left nephrectomy was performed and the tumor was fixed in the 4% formol. The patient did well postoperatively and no recurrence was detected 6 months after the surgery. The patient had no signs of tuberous sclerosis.

PATHOLOGY

The kidney measured 12,5x9x3 cm. Gross examination of the kidney revealed well circumscribed and encapsulated tumor located in the central parts of the kidney measuring 8x8,5x3,5 cm. The renal pelvis and hilus of the kidney was not involved by the tumorous mass. The tumor cut surface was light brown to yellow with well visible grey bundles and foci of the metaplastic bone formations. Small cysts were noted. No necroses were observed in the tumor.

Microscopically, the tumor characterized an intimate intermixture of epithelial cells and smooth muscle tissue. Epithelial parts were composed of sheets of cells with small round basophilic nuclei, scant cytoplasm, and well-defined cell borders. The tumor cells were mainly arranged into the tubular formations or in some areas the lumina of the tubules were collapsed so that they reminded a solid growth. In some parts the epithelial cells in adenomatous tubular formations were endowed with clear snouts. These clear snouts had a blister-like quality and they grew as though grafted on the secretory cells lining the tubules. No atypias and pleomorphism were present throughout the tumor and mitoses were absent. The muscular component consisted of poorly cellular, leiomyomatous bundles with eosinophilic cytoplasm and elongated nuclei. It encircled the whole tumor and intimately intermingled with the epithelial component. Focally the leiomyomatous bundles formed abortive vessels, which had incomplete and irregular wall and lacked elastic layer. The leiomyomatous stroma focally underwent a myxoid to hyalinized change with a rare metaplastic ossification. Thin and disorganized leiomyomatous bands ran throughout the myxoid areas of the tumor in many fields. No adipose tissue was seen in the leiomyomatous stroma. Even more interesting was a peculiar vascularization of the tumor. All epithelial component in solid and adenomatous tubular arrangement revealed an intimate association with small capillaries. A fine labyrinth of the capillaries, which ran on the outer circumference of the adenomatous structures, rimmed the rows of the epithelial cells. Such arrangement was inconspicuous in hematoxylin and eosin staining and it was best apparent in immunohistochemical staining with smooth muscle antibody, which revealed the pericytes of the capillaries adjacent to the epithelial component. Similar meshwork of blood capillaries was observed in staining with antibodies to Ulex Europeus or CD31, which marked the endothelial cells of the capillaries. We have not found a single epithelial cell structure in the whole tumor unassociated with these tiny blood capillaries. Interestingly, rare single adenomatous structures in the parts of the stroma, with myxoid change was often so richly surrounded by capillaries that an illusion of a gland lying inside of a capillary was sometimes generated.

Immunohistochemically the epithelial component of the tumor stained with the antibodies to cytokeratins (AE1-AE3, CAM5.2) and EMA and was negative with all the rest of the antibodies. Leiomyomatous stroma was positive with the antibodies to actin and desmin and was negative with the rest of the antibodies including HMB-45. MIB1 was practically negative in the tumor cells (1 positive cell per 10 HPF) pointing to a very low proliferation rate of the neoplasm.

Ultrastructurally, the tissue was damaged considerably by a long storage in the formol. Each cell had an irregularly clefted nucleus with very inconspicuous nucleolus. The cells were attached to each other by well-formed desmosomes. The cytoplasm was rich in organelles, which included a smooth endoplasmic reticulum, mitochondria, lysosomes and Golgi apparatus. The smooth endoplasmic reticulum often contained densely stained bodies. The cytoplasm of the tumor cells had clear snouts, which were edematous, and seemed to be poor in organelles. The cytoplasm formed microvilli, which were particularly well developed in the space between the clear cytoplasmic snouts. The spindle cell mesenchymal component of the tumor had ultrastructural evidence of leiomyomatous differentiation. It consisted of cells with cigar shaped indented nuclei, and inconspicuous nucleoli. The cytoplasm of these cells had well-developed endoplasmic reticulum and it was packed with intermediate filaments. The bundles of microfilaments often grouped together to form dense bodies. No striated structures suggestive of melanosomes as sometimes seen in renal and extrarenal angiomyolipomas were observed in the leiomyomatous component of the tumor.

DIAGNOSIS

Angiomyoadenomatous tumor of the kidney (AMET) (1).

COMMENT

In the literature there are reports, describing adult patients with renal neoplasms, harboring epithelial and stromal components within one tumor mass as for example renal cell carcinomas surrounded by angiomyolipoma (3,4). Such neoplasms, which have epithelial and mesenchymal components, are extremely rare in the human pathology. We are aware of another tumor consisting of epithelial and stromal tissues in the kidney. Michal et al published a tumor, which they called benign mixed epithelial and stromal tumor of the kidney (8,9). Histologically the adenomatous part of the tumor was composed of ducts with well-formed lumina. The largest ducts contained cells with hobnail appearance. The cytoplasm of the cells of the ducts stained deeply red with eosin stain. The adenomatous component was accompanied by a characteristic mesenchymal stroma. It was composed of well-differentiated spindle cells closely resembling ovarian stroma or it was similar to the arrangement of the spindle cells in the solitary fibrous tumor. This ovarian-like stroma was always centered on the adenomatous component and the stroma never formed compact sheets devoid of adenomatous differentiation (8,9). This benign mixed epithelial and stromal tumor of the kidney published by Michal et al (8,9) is identical to the tumor of the kidney published by Adsay et al (10). Examples of other biphasic tumors in the kidney are mesoblastic nephroma of adulthood (10) and nephrogenic adenofibroma (12). In organs outside the kidney such examples are cystadenomas and cystadenocarcinomas of the pancreas and liver having spindle cell ovarian-like stroma (13,14), occurring exclusively in women and a strange benign tumor consisting of epithelial and stromal components of the thyroid reported recently. AMET differs in many aspects from all these renal and extrarenal tumors. The stroma of AMET consists of mature leiomyomatous bands without adipose tissue, which often formed abortive angiomatous structures. It is entirely different from the ovarian-like stroma (8,9,13,14) and angiomyolipoma (3,4). In contrast to all other

tumors published in the literature the adenomatous component of AMET has an interesting intimate association with the blood capillary vessels. A labyrinth of fine capillaries, which runs on the outer circumference of the adenomatous structures, rims the rows of the epithelial cells. Such arrangement was best apparent in staining with smooth muscle antibody, which revealed the pericytes of the capillaries adjacent to the epithelial component or with antibodies to Ulex Europeus and CD31, which marked the endothelial cells of the capillaries. We have not found a single epithelial cell structure in the whole tumor unassociated with these tiny blood capillaries. Such intimate association of the tubules with capillaries reminded of a caricature of the normal nephron where an intimate relationship of the tubules to the capillary blood vessels is seen.

In differential diagnosis AMET must be distinguished from renal cell carcinomas. In difference from them we have not found any mitoses (both typical and atypical) or pleomorphism. We are not aware of any type of renal cell carcinoma associated with leiomyomatous stroma. Clear cell snouts, which represented a typical light and electron microscopic sign of the cells in AMET have not been so far described in any benign or malignant renal tumors. Even if rich capillary network is a feature of many renal cell carcinomas, such intimate relationship of the capillaries accompanying practically all tubules of our case differentiates our case from any other renal cell tumors.

Because of the peculiar arrangement of the epithelial, leiomyomatous component and the unusual relationship of the vasculature and the tubules of the epithelial component we think AMET defies any current classification scheme of the renal cell neoplasms. Owing to very low proliferation of the tumor as revealed by antibody MIB1 (1 cell per 10 HPF positive) we think that the tumor is going to behave, in spite of its size, in a benign way.

In summary we present a unique tumor composed of an admixture of benign epithelial and leiomyomatous components found in nearly 7000 cases of renal neoplasms seen in our tumor registry. The epithelial component revealed intimate relationship to the fine structure of the capillaries caricaturing thus normal nephron where an intimate relationship of the tubules and capillary blood vessels is seen.

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QUESTIONS

1. Does the myoid part of the tumor belong to the PEComas? **Yes or No.** ____.
2. Is the tumor related to the syndrome of the tuberous sclerosis? **Yes or No.** ____.

3. Appartiene alla famiglia dei "PEC-oma la componente mioide del tumore "?
4. **Si o No.** ____.
5. Il tumore in questione è correlato alla sclerosi tuberosa? **Si o No.** ____.

CASE 16 -28884-1

Michele Bisceglia, M.D., Department of Pathology, IRCCS-Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy

CLINICAL HISTORY

A 22 year-old female patient was admitted complaining for one year of a left-sided abdominal pain. On computed tomography scan and magnetic resonance imaging a mass lesion of 5 cm in size was disclosed in the left adrenal gland. The patient did not show any clinical features due to hypercortisolism nor to sex steroid or mineral-corticoid production, with normal arterial blood pressure (120/80 mmHg), normal body fat distribution, no "striae rubrae", regular menses, and normal hair distribution. However hormone biochemical assays showed the following abnormal test values: urinary free cortisol of 74.2 and 109 μg/dl in two separate determinations (n.r. 8-60); plasma cortisol level 8.2 μg/dl not suppressed by dexametazone 1 mg overnight administration (n.r. <2.5); fasting ACTH plasma levels (three samples) of 8-4.9-4.47 ng/ml (n.r.10-60). Other pertinent hormone plasma and urinary values were in normal range (aldosterone= 40-393 pg/ml – clino/orthostatism; plasma renin activity= 0,17 - 0,68 ng/ml/h –clino/orthostatism; urinary epinephrine= 10 μg/24hours; urinary norepinephrine= 70 μg/24hours). On a clinical evidence of a tumor lesion with (asymptomatic) autonomous cortisol production the patient underwent a left laparoscopy adrenalectomy. During this surgical procedure the patient experienced significant variations of her arterial blood pressure.

PATHOLOGY

GROSS FINDINGS

Grossly a well circumscribed and solid tumor, measuring 6 cm in greatest dimension and weighing 42 gr, was present in the adrenalectomy specimen, which on sectioning appeared dark tan to brownish in color.

MICROSCOPICAL FEATURES

Histologically the tumor was exclusively composed of large eosinophilic cells, many of them with atypical nuclei, some smudgy and hyperchromic, others with large nucleoli, and a few with intranuclear inclusions. Some cells were of huge size and some multinucleated. The cells formed irregular groups and sheets qualifying a main diffuse architectural pattern. The tumor was encapsulated and well delimited from the residual adrenal gland. However a couple of figures of capsular invasion have been observed in a section (other than the one distributed). None of the sections showed mitotic activity or tumor cell necrosis. No vascular or sinusoidal invasion was seen. Immunohistochemically the tumor was positive for cytokeratins (MNF-116 and Cam 5.2),
MelanA antigen (MART-1 MoAb), synaptophysin, neuron-specific enolase, and mitochondrial antigen (113-1MoAb). No immunoreactivity was detected for vimentin, S-100 protein, chromogranin, HMB-45.

ELECTRON MICROSCOPICAL FINDINGS

The ultrastructural analysis was performed on a tissue fragment which was retrieved from the paraffin embedded material. In the tumor cell cytoplasm the main feature was represented by numerous and compactly arranged mitochondria, some of which displaying tubulo-vesicular cristae. Short stacks of rough endoplasmic reticulum also were seen while no dense-core membrane-bound granules were found. On the cell surface an investment by basal lamina and some well-formed intercellular junctions were also documented.

DIAGNOSIS

Borderline oncocytic cortical tumor of the adrenal gland (oncocytic cortical tumor of uncertain malignant potential).

DISCUSSION

Oncocytic tumors are rare neoplasms, histologicaly comprised of epithelial cells with abundant acidophilic, granular cytoplasm that can be arranged in alveolar, tubular, or solid patterns. They are usually defined as tumors predominantly or exclusively composed of oncocytes. Electron microscopic studies of these tumors have shown that the cytoplasm of oncocytes is packed with mitochondria (1). Oncocytomas have been described in many organs, such as the kidney, the salivary gland, endocrine glands, and also in a variety of other anatomical sites (1-2). Although the first description of a case of oncocytic tumor of the adrenal gland dates back to one and a half decade ago (3), the concept of a true oncocytoma in this site has not been widely popularized yet. Oncocytic tumors originating from the cortical adrenal gland are exceedingly rare, with 36 total cases so far reported (3-19), a good deal of which only in abstract form (9, 19). One case diagnosed on FNA and fully reported (20) has not been included in this review since it likey appears also in a series which the same authors subsequently presented elsewhere (19)

Obviously the most important practical problem revolving around this tumor type –as any other type- regards its biologic behaviour. The biologic behaviour and the clinical outcome of adrenal tumors *in general* are difficult to predict, this statement being valid not only for medullary tumors (pheochromocytomas –for which the aphorism has been coined that "nothing but metastasis is a reliable sign of malignancy") but also for cortical tumors. Tumor size and weight has been long emphasized as a predictor of biological behaviour with large tumors (>100gm and >5cm) predicted of lethal outcome and –conversely- small tumors (>50gms and >5cm) expected to run favourably. However since it was not the rule (in ref. 20: cf. -refs 3,4,20,22-24), a number of systems have been devised in order to distinguish benign from malignant adrenocortical tumors of the conventional category (*non-oncocytic*) (21-25), of which the most widely used and reliably accepted system being the Weiss system (23). **In this system a combination of nine criteria** (nuclear grade III-IV, mitotic rate >5per 50 HPF, atypical mitoses, clear cells ≤25% of the tumor, diffuse architecture, necrosis, venous invasion, sinusoidal invasion, capsular invasion) are taken into account with ≤2 positive criteria meaning benignancy and ≥4of them meaning malignancy, respectively. In that paper the patronymic author also considered the practical possibility that <<there must be rare adrenocortical tumors that fall within the border zone of this classification system>> (23) supposedly alluding to the 3 scored cases. However, the treshold of the divisional scoring system was further lowered so that the 3 scored cases were also included in the malignant side of the category (25-26). Further there are also authors, included Weiss hiself (26), who give precedence to some features over others, who would probably warrant a diagnosis of malignancy in presence of high mitotic activity, atypical mitoses, and venous invasion even in isolation (which should merit be called *major criteria*), although they suggest to look for the other two criteria. Around a

decade ago Sasano et al (4) reported on a small series of *adrenocortical oncocytic tumors* and –despite the short available follow-up- first opened the question about the biological behaviour of this subset of neoplasms and wondered whether the Weiss system was also valid for them. Quite recently with a contribution of a series of 7 new cases and a review of the previous literature, Lin et al (12) suggested that **the Weiss system be modified for the assessment of adrenocortical oncocytic tumors**. For oncocytic neoplasm of the adrenal cortex, the morphologic (microscopical) criteria of malignancy employed for conventional adrenocortical neoplasms do not apply. By definition oncocytic tumors would be scored with 3 positive criteria and thus designated as malignant, which is not supported by the available follow-up of the so far reported cases (up to 5-8 years in some of them). In this article by Lin et al of 7 cases of oncocytic tumors (5 benign oncocytomas, 2 of uncertain malignant potential) the suggestion is given to make distinction between benign oncocytomas and possible malignant oncocytomas on the basis of mitotic activity, necrosis, and invasion(-NOS). However it has not been stated in there how many criteria are needed to warrant a diagnosis of malignancy (i.e. "no concrete suggestions"). The two tumors of uncertain malignant potential were so designated due to the presence of necrosis and/or mitotic activity.

Most recently Wu et al (19) who have presented a series of such cases (5 oncocytic tumors with uncertain malignant potential and two oncocytic carcinomas) did not explain which the combination of their criteria was in order for them to classify their tumors in a three-tiers based system (oncocytomas, oncocytic tumors with uncertain malignant potential, and oncocytic carcinomas).

I personally contacted both dr. Weiss and dr. Ordonez at this regard. I received answer from dr Weiss only: here the rules for his personal usage <<if an oncocytic tumor has mitotic rate >5/50, atypical mitoses or venous invasion è [(i.e. one of those *major criteria*)] the oncocytic tumor is malignant>>, <<if the tumor has one of the other worrisome features (large size, necrosis, or capsular invasion) [(i.e. conversely eligible as *minor criteria*)] the tumor is defined of uncertain malignant potential>>, if <<it has no one of the worrisome features it is reasonable to call it benign>>. The analysis of this response should make one be wary about the size of an oncocytic tumor, as an important parameter in the assessment of the prognostic prediction. In summary, concerning with the issue of oncocytic tumors, from the analysis and the interpretation of the available published data, and for the lovers of scoring systems and classifications we probably could say that if an oncocytic tumor has one of those major criteria (high rate of mitotic activity, atypical mitoses, venous invasion) the tumor should be regarded as malignant, if it has one of the minor criteria (large size, necrosis, or capsular invasion), then it should be considered borderline, if it has no one either of the major and minor criteria (still having the definitional characteristics of high nuclear grade, non-clear cell composition, diffuse architecture), then it should be called benign. Anyhow many open questions remain and referring to this tumor area it sems that *pathology is more like driving than a science.*

The oncocytic tumors so far reported have been described of variable size (3-20 cm) and weight (12-2415 gm), with significant female to male predominance (2.5:1), wide age range (27-71), and a strong left to right side prevalence (3.5:1). Out of a total of 36 tumors, 24 have been designated as benign, 5 malignant (5,15,17,19), 7 borderline (12,19). For the vast majority they were nonfunctioning, with only three hormonally active (one case with virilization [6], one case with feminilization [18], one case with Cushing's syndrome [15]) and this probably explaining the large size commonly attained by these tumors (in 18 cases: median size 8 cm; median weight 205 gm). Immunohistochemistry (12,16,17,19): positive markers: –cytokeratin 18, synaptophysin, Melan-A (MART-1), mitochondrial antigen (mES-13,), neuronspecific enolase, inhibin, CD10, vimentin; negative markers:

cytokeratin 20, S100 protein, HMB-45, epithelial membrane antigen, p53, (usually) steroidogenic enzymes; MIB-1/Ki67 useful in the assessment of benign case versus borderline and malignant (12,29). Electron microscopy (4-6,12,16,18): ten out of a total of 25 fully reported cases were so studied with ultrastructural cell findings of oncocytes. Problems in diagnosis: to distinguish true oncocytic tumor cells versus (pseudooncocytic) compact cells of conventional adrenocortical carcinomas; adequate tumor sampling to avoid overlooking areas with necrosis or mitotic activity, which can be focal; to follow strict criteria for diagnosis and differentiation. Differential diagnosis: oncocytic pheochromocytoma (28); low-grade adrenocortical conventional carcinoma exclusively composed of compact cells; involvement from conventional renal carcinoma with oncocytic features or from eosinophilic variant of chromophobe renal carcinoma; hepatocellular carcinoma on FNA cytology (personal unpublished observation). Aids in diagnosis: employement of immunohistochemistry (27,29) and electronmicroscopy, especially in dubious cases.

Follow-up: At the last control –ten months after the surgical intervention- the patient is alive and without evidence of disease.

CONCLUSIONS:

Although it seems apparent that the majority of neoplasms are benign (12,18), still the follow-up generally short as well as the small number of the cases in overall so far reported suggest caution and further larger studies and longer follow-up before definitive conclusions are drawn (19,30).

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QUESTIONS

1. According to the Weiss microscopic system which are the nine criteria employed for differentiating benign conventional (non-oncocytic) adrenocortical tumors from malignant?
   List all of them: .....
   .....
   .....
2. Should the Weiss system be modified for the evaluation of oncocytic adrenocortical tumors? **Yes or No.** ____.

1. Quali sono i nove criteri microscopici utilizzati nel sistema di Weiss per differenziare tumori benigni corticosurrenalici convenzionali (non-oncocitici) da quelli maligni?
   Elencarli tutti: .....
.....
   .....
2. Dovrebbe il sistema di Weiss essere modificato o no, quando si tratta di applicarlo a tumori adrenocorticali di tipo oncocitico? **Si o No.** ____.

CASE 17. M25927/94

Michal Michal, M.D., Sikl's Department of Pathology, Charles University, Medical Faculty Hospital, Pilsen, Czech Republic.

CLINICAL HISTORY

A woman aged 32 years and presented with signs of highly elevated plasma and urine levels of cortisol and elevation of plasma levels of aldosterone and a highly elevated urine level of aldosterone was observed. Concentrations of adrenaline (50x) and noradrenaline (10x) highly exceeded the normal levels. The patient suffered from secondary hypertension associated with hypokalemia, metabolic alkalosis the diabetes mellitus. The patient complained of gaining weight muscle weakness, rounded faces, purple abdominal striae, edemas of lower extremities and polyuria. All these symptoms disappeared after the surgical excision of the tumor and the patient are well and without recurrences five years after the removal of the tumors.

Grossly the tumor was 9x7x5 cm in size and well encapsulated. It had a yellow color and a diffuse, solid consistency. There could be seen two components in the tumor mass. One component was eosinophilic and it stained immunohistochemically positive with antibodies to Melan A and chromogranin and cytokeratin negative. The other component was cyanophilic and it was immunohistochemically strongly chromogranin positive and Melan A and cytokeratin negative. The eosinophilic component was identified to be of cortical adrenal origin and the cyanophilic component was represented by pheochromocytoma.

DIAGNOSIS

Corticomedullary tumor of the adrenal glands (CMT) (Michal M., Havlíček F.: Corticomedullary tumors of the adrenal glands. Pathology Research and Practice 1996:192: 1090-4).

COMMENT

Pheochromocytoma is known to give rise to several types of composite tumors. There are described combinations of tumors consisting of pheochromocytoma with ganglioneuromas, neuroblastomas (1,4,6,11,13,18,22) or spindle cell sarcoma (9,16,17) in the literature. Rarely, pheochromocytoma is associated with a separate adrenal cortical adenoma (3,10,21). There are described in the literature a few unique cases of CMT consisting of nests and cords of the pheochromocytoma and cortical adenoma growing within the same tumor mass (2,14,24).

The picture of the interweaving nests and cords of the adrenal cortical and pheochromocytoma cells of the CMT might resemble the interface between hyperplastic cortical cells of the residual adrenal cortex and pheochromocytoma sometimes seen in common pheochromocytomas. In common to the pheochromocytoma the tumorous cells can invade the surrounding residual cortex of the adrenal gland for a certain distance (12,19) and the light microscopical picture in these parts might resemble the CMT. However, the cortical adenoma tissues CMT permeates the whole thickness of the pheochromocytoma component and the total weight of the cortical adenoma cells reached several tens of grams, exceeding many times the normal weight of cortical cells. It excluded the possibility that the cortical cells CMT would be nontumorous residual cortical cells permeated by the pheochromocytoma.

The cortical adenoma component of the CMT might be difficult to be distinguished from the pheochromocytoma cells. However, the cortical adenoma cells were more

homogeneously eosinophilic with more accentuated cell borders than the pheochromocytoma cells, which in turn are usually more cyanophilic. In addition, the cells of the pheochromocytoma component contained occasional pleomorphism, intranuclear cytoplasmic inclusions (5) and intracytoplasmic, hyalin, PAS positive globules (13). These inclusions and globules are absent in the adrenal cortical cells. Notwithstanding, focally the pheochromocytoma component revealed lipid degeneration (20,23) and the differences between the two components may became very subtle. Distinction between these two components could be, however, made immunohistochemically. Antibodies to synaptophysin and chromogranin A stained only the pheochromocytoma cells and antibody to EMA stained only a proportion of the cortical adenoma cells. Cytokeratin antibodies failed to stain the tumor entirely. The negativity of the cortical adenoma component with the antibodies to cytokeratin should not be surprising, because, despite the biochemically documented presence of cytokeratin intermediate filaments in these tumors, significant proportion of the adrenocortical tumors will not label with anti-cytokeratin antibodies in formaldehyde fixed tissue due to the deleting effect of fixation (7,15).
The histogenesis of the CMT is difficult to determine. A question whether the cortical adenoma and pheochromocytoma components of CMT grow independently or whether their growth is interrelated is difficult to resolve. Pheochromocytoma can rarely produce ACTH (8) and a possibility exists that this ectopic ACTH production might initiate the cortical adenoma component of this tumor. The extreme rarity of the published cases of CMTs suggests that CMT might represent one of the „collision" tumors.

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Case 17. QUESTIONS for CME assessment.

1. Could the Cushing's syndrome be caused by pheochromocytoma component of the corticomedullary tumor? **Yes or No.** ____.
2. Has been the cortical component always benign in the described cases of the corticomedullary tumor? **Yes or No.** ____.

1. Potrebbe la sindrome di Cushing essere causata dalla componente di feocromocitoma del tumore corticomidollare? **Si o No.** ____.
2. Si è sempre comportata in modo benigno la componente corticale del tumore corticomidollare? **Si o No.** ____.

SURGICAL PATHOLOGY -SESSION V. HEAD & NECK

CASE PRESENTATIONS (5 cases)

Case 18. Diffuse sclerosing variant of papillary thyroid carcinoma associated with clinical signs of Hashimoto's disease

(D. Ben-Dor)

Case 19. Synovial sarcoma of parapharyngeal area

(G. Falconieri)

Case 20. Epithelioid angiosarcoma of the thyroid

(J. Lamovec)

Case 21. Clear cell myoepithelial carcinoma of the salivary gland

(M. Michal)

Case 22. Endolymphatic sac papillary tumor -Heffner Tumor

(M. Bisceglia)

CASE 18. 3368-01

David Ben-Dor, M.D., Department of Pathology, The Barzilai Medical Center, Ashkelon, Israel

CLINICAL HISTORY

The patient is a 23 year old female in good health who presented to the hospital endocrinologist for investigation of a painless enlarged thyroid. There were no particular clinical complaints. Physical examination revealed an overall fairly diffuse twofold enlargement of the thyroid, apparently with some predominance on the right side with no particular nodularity. Lymph nodes were not palpated. The initial impression was Hashimoto disease. The patient was clinically euthyroid; TSH taken at the time was normal. A fine needle aspirate was performed on the right lobe. (Subsequently received serology showed elevated anti-thyroid globulin).

CYTO- AND HISTOPATHOLOGY

The FNA was remarkable for scattered psammoma bodies, and clumps of atypical epithelial cells on a lymphoid background . The opinion given was suspicious for tumor, with recommendation for surgical removal of the lobe.

The lobe was removed and sent for frozen section. It measured 5x3x1 cm. and contained a discrete nodule 0.7 cm diameter. The remaining parenchyma was colored yellowish light tan (in contrast to the usual mahogany red coloration) and felt slightly infiltrated. Frozen section of the nodule showed the classical diagnostic features for papillary thyroid carcinoma. A second frozen section from the parenchyma outside the nodule showed clumps of atypical cells containing psammoma bodies and multiple lymphoid follicles.

Completion thyroidectomy was directly performed. The left lobe measured 5x2.5x1.5 cm and was also noted to be of increased consistency. Another piece of tissue containing pretracheal lymph nodes was also sent.

Examination of sections from both lobes (the sections used to make the blocks contributed to this demonstration are from the left lobe which were decalcified prior to submission) on scanning magnification shows abundant reactive lymphoid follicles. At this power the architecture of the gland doesn't seem much disturbed: the parenchyma is subdivided by sclerotic bands and contains some distended follicles, but overall it appears largely preserved. As the magnification is increased, one begins to see scattered groups of psammoma bodies contained in irregular cell aggregates. On high magnification, these aggregates are clumps of neoplastic cells which are enlarged and rounded to the with squamoid features . The nuclei are large and very pale but contain a little punctate residual chromatin. True intranuclear inclusions are not seen. Many of these tumor aggregates are situated in the fibrous bands and lay in clefts characteristic of lymphatic spaces. The bulk of the surface area of the sections are occupied by thyroid follicles. Some of these are distended and lined by cuboidal cells without atypia and appear to be residual normal structures. However alongside and surrounding them are smaller more irregular follicles lined by cells with more ample eosinophilic cytoplasm and enlarged nuclei of varying atypia paler than usual but without the classical incontrovertibly diagnostic features necessary to define them with confidence as neoplastic. Aside from the prominent lymphoid follicles, there are abundant lymphocytic and plasmactyic infiltrates seen under

higher power. Four small lymph nodes (two submitted separately as pretracheal nodes and two found in the lobectomy specimens) contained small tumor metastases.

DIAGNOSIS:
Diffuse sclerosing variant, papillary carcinoma of the thyroid (bilateral).

DISCUSSION
The clinicopathologic features of this entity are nicely delineated by John Chan (1). In addition various other articles describe series of patients, amongst them (2) and (3). This is a distinct variant of papillary thyroid carcinoma often occurring in young females. In some cases a clinical suspicion of Hashimoto disease arises. What sets this entity apart is its appearance in a diffuse fashion in either one or both lobes often without localizing features in a background suggestive of inflammatory thyroid disease. As Dr Chan enumerates, these are the additional features that are characteristic of this subtype: sclerosis, heavy lymphoplasmacytic infiltrates, abundant psammoma bodies, scattered islands of papillary carcinoma with squamous features, and extensive lymphatic permeation. The case demonstrated here shows these features and is thus consistent with the entity. Often patients have lymph node metastases (as does this patient) as well as evidence of distant spread (not proven in this case). Despite these, these patients do not do worse than patients with the classical type of disease.

There are some aspects of the histology demonstrated in this case that are not necessarily addressed by the standard list of criteria. The fibrosis in this case is mostly limited to the presence of fibrous bands dissecting through the lobe. Apparently there is no minimal amount necessary but it can be quite extensive. Aside from the obvious presence of tumor islands diagnostic for this condition the parenchymal follicular architecture is mostly intact. One can question the nature of some of these follicles as to whether they represent tumor (i.e. follicular variant of papillary carcinoma) or Hashimoto disease. While some of the follicles be normal or atrophic, many are smaller and atypical. While there is some possible nuclear cytomorphological overlapping between these elements and the tumor cells the latter show atypia in excess of what is found in the follicles.. The cytoplasm of the follicular cells doesn't show neither the abundance or the bright eosinophilia characteristic of Hurthle cells. I think that these follicles aren't malignant and don't meet the diagnostic criteria for Hashimoto disease (despite the positive serology). These altered follicles may express changes reactive to the inflammation. (Note: slides were previewed by some members of the group including Dr John K C Chan and these comments reflect their opinions). Only one of the 10 cases described in (3) showed histological features of Hashimoto disease while this was not indicated in any of the 15 cases described in (2). In (3) the tumor in some of the cases showed partial follicular architecture while this is not mentioned in (2). From my understanding aside from the diagnostic attributes as listed above, the nature of the background thyroid parenchyma and the degree to which it is preserved or obliterated by the sclerosis is not cardinal . While serology was not mentioned in one series(2) in the other 3 of the patients were positive. Thus to what extent this entity truly arises on a background of Hashimoto disease which can be documented objectively is not clear.

The interest of this entity derives from its confounding nature: the lack in many cases of an identifiable mass along with clinical features suspicious for Hashimoto disease. This could make the diagnosis of tumor difficult at the onset and possibly delay its recognition. The presence of diffuse glandular enlargement with the appropriate clinical findings would

make it very inviting to diagnose inflammatory disease and leave it at that. I wonder to what extent FNA is successful in general in picking the tumor up due to it being so scattered and focal often on a background of extensive generalized sclerosis. In this case the diagnosis was facilitated by previous FNA (whose success in this instance may be attributed in part to the relatively minor fibrous changes) which led to surgery. Even so we were "fortunate" in that a focus of consolidated tumor was found which made the confirmation on frozen section easier; to make such a diagnosis in the absence of any mass might be a trying exercise, especially as a frozen section. At the other end after the nature of the pathology is recognized a different problem arises: the spread of tumor might lead one to attach a prognosis less favorable than that of the usual thyroid papillary carcinoma. In fact it is accepted that despite the tendency of this tumor to spread outside the thyroid the prognosis is in fact not worse, as patients survive despite the disease. Parenthetically, the profusion of psammoma bodies brings to mind a possible parallel with a different entity, serous ovarian psammomacarcinoma which is known to be an indolent tumor. Thus knowledge of this entity provides reassurance to the clinician and the patient despite the presence of findings which would be ominous in other contexts.

POSTSCRIPT: patient continues to be in general good health a little over a year following diagnosis.

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QUESTIONS

1. In the name of the discussed entity, diffuse sclerosing variant, the term diffuse refers to:
   - **A.** The extent of sclerosing changes.
   - **B.** Diffuse distribution of the tumor.

   ***Mark the appropriate letter.**
2. The presence of distant spread and lymph node metastases in a young woman with thyroid cancer is always a bad prognostic feature. **True or False.** ____.

1. La dizione "variante sclerosante diffusa" attribuita al tumore in questione si riferisce:
   - **A.** Alla estensione della sclerosi.
   - **B.** Alla distribuzione diffusa del tumore stesso.

   ***Contrassegnare la lettera giusta.**
2. La presenza di una diffusione a distanza e la presenza di metastasi linfonodali in una giovane donna con cancro della tiroide è sempre un segno di cattiva prognosi. **Vero o Falso.** ____.

CASE 19. 01-6865.

Giovanni Falconieri, M.D., Department of Pathology & Laboratory Medicine, division of Anatomic Pathology, "S. Maria della misericordia" General Hospital, Udine.

CLINICAL HISTORY

A 21 year-old man complained of progressive swallowing discomfort and sense of throat fullness. Physical inspection revealed a firm mass of the deep soft tissue of the right lateral aspect of neck. A Cat scan confirmed the presence of a solid mass located in the right parapharyngeal space. Other routine investigations as well as principal laboratory determinations were in the normal range. There was no further evidence of cervical lymph node enlargement. On direct inspection, there was no mucosal lesion within the upper airways.

PATHOLOGY

GROSS FINDINGS

The specimen measured 7 x 4 x 3 cm and consisted in a lobulated, rubbery mass covered by a diamond-shaped portion of pharyngeal mucosa. Cut surface was gray-tan, homogeneous, with inconspicuous regressive changes.

HISTOLOGIC FINDINGS

Routinely stained sections showed a biphasic tumor composed of haphazardly admixed fascicles and tubulo-glandular epithelioid structures. Tumor fascicles were composed of spindle cells having tapered-end nuclei and scant cytoplasm, whereas the epithelioid areas features usually tall to cuboidal cells lining up solid or empty spaces. In contrast to spindle cells, their nuclei had an open chromatin. Mitotic activity, though in a low rate, was present

IMMUNOHISTOCHEMICAL PROFILE

The spindle cells were strongly reactive for vimentin and negative for epithelial markers. The epithelioid cells exhibited a complementary immunostaining pattern, with positivitity for both keratins (AE1/3, Cam 5.2, CK7, CK19) and EMA and negativity for vimentin. No immunoreactivity was noticed in both components for actins, desmin, CD34, S100 protein, calretinin

DIAGNOSIS

Biphasic synovial sarcoma of the parapharyngeal soft tissues.

DISCUSSION

It is estimated that about 10% of soft tissue lesions occur in the head and neck, the majority of which being benign or reactive conditions affecting children or adolescents. True sarcomas are rare and account for less than 1% of soft part lesions observed in adults. Nevertheless a substantial proportion of specific histotypes including rhabdomyosarcoma, angiosarcoma, malignant peripheral nerve sheath tumors seem to have more propensity to occur in the head and the neck than in other locations. [2,5,10,13] Etiopathogenesis is largely speculatively, although Roentgen therapy for previous upper respiratory tract neoplasms has been advocated as a possible causative factor.[15,16] Except for angiosarcoma, which has predilection for the scalp of elderly individuals, most sarcomas arise within the deep neck soft tissues. They are usually large at presentation, thus making radical resection difficult.

Adverse prognostic factors are residual tumor, size > 5 cm, and older age.[5] However, the cumulative survival rate (all histotypes) at 5 years is 60%.[2]
Most synovial sarcoma (SS) are located in the limbs, often close to major joints, and in the trunk. A minor but not irrelevant percent occur in the neck, accounting for less than 10% of reported cases[3,6,11,13]: the majority arose from the pre-vertebral and parapharyngeal space, but reports are available describing it at virtual any anatomic sites in the cervical area including the larynx and hypopharynx.[3,6] According to various series, the patients are often adolescents or young adults, and are usually younger than those with SS at other sites.[6,11]
The most frequent patient's complaints are swallowing and breathing difficulties or throat hoarseness. Pain is reported in 20% of cases. For this reason, patients usually seek early medical attention and receive prompter therapy than those with limb or trunk lesions. Nevertheless, about one third of patients experience local recurrence and one fourth have metastatic tumor at presentation or during the follow up.[6] Younger age, a low mitotic count and a large proportion of calcifications and ossifications apparently correlate with a better outcome.
Head and neck SS usually present as an ovoid, lobulated and rubbery mass. Lesions as large as 12 cm have been reported.[11] Hemorrhage and necrosis may be seen on inspection of cut surface. Cystic changes may be occasionally noted. There seem to be no specific histologic feature of cervical SS when compared to the homologues of limbs and trunk.

DIFFERENTIAL DIAGNOSIS

The diagnosis of SS is traditionally based on the recognition of the classic biphasic tumor pattern made up of epithelioid, cubic cells arranged in pseudoglandular structures haphazardly admixed with disorderly fascicles of swirling spindle cells. Either the spindle or epithelial component may predominate, although pure epithelioid SS are much more rare. A ground substance rich in thick fibrous collagen is often encountered; scattered calcifications (with or without ossification) may be frequently seen. Immunohistochemistry may strengthen the microscopic interpretation although the antigen profiles emerging from antibody panels lack the specificity required for diagnostic confirmation. SS is usually positive for keratins (especially CK7 and CK19) and EMA, the epithelioid component being more consistently reactive than the spindle cell foci. A complementary staining pattern is seen with vimentin. Other antibodies often reported positive in SS are S100 protein, bcl-2 as well as Leu 7.CD99 is expressed by a sizable proportion of SS but its practical usefulness, as Dei Tos et al pointed out[4], remains largely questionable.

The differential diagnosis of SS entails several conditions including metastatic tumors to the head and neck (A) and distinct soft tissue malignancies (B)

A. As a general rule, any monophasic sarcomatoid or biphasic tumor of the cervical soft tissues must be distinguished first from a metastatic lesion especially from primary epithelial tumor of the upper aerodigestive tract or major salivary glands. Soft tissue extension from a ruptured metastatic lymph node is a rather common occurrence in squamous cell carcinoma. Clinical history is generally more enlightening than histology although epidermoid characteristics are focally retained in pleomorphic or sarcomatoid forms. When compared to SS, spindle squamous cells are more atypical and also exhibit more consistent immunopositivity for keratins. Metastatic adenocarcinoma especially originating from the salivary gland may have a sarcomatoid differentiation in metastatic site thus being difficult to separate from monophasic SS.[1] History of previous surgery and, again, diffuse immunoreactivity of poorly differentiated carcinoma for keratins help in the correct recognition. Melanoma is known to have an extraordinary capability of simulating

a wide spectrum of malignant tumors, including sarcomas, an should be always suspected when dealing with a malignant tumor of neck soft parts. History of previous surgery for pigmented skin lesion is obviously a useful hint, however primary melanoma may not be clinically apparent if located within the nasal mucosa, eye, meninges. Metastasis can be also the first sign of a regressed cutaneous tumor. Generally, a strong and diffuse immunostaining of both nuclei and cytoplasms for S100 protein strongly favors malignant melanoma. HMB45 positivity is confirmatory but a negative staining is not as meaningful since poorly differentiated melanomas often do not express melanin antigens. Metastases of biphasic or sarcomatoid mesothelioma may be difficult to distinguish from SS on a pure morphologic ground. Interestingly, rare cases of mesothelioma initially manifesting with extra-thoracicmetastases have been reported.[14] However patients with mesothelioma are older that those with SS. More important, metastatic mesothelioma is usually of epithelial type.

A number of soft part malignancies enter the differential diagnosis of biphasic and especially monophasic spindle cell SS. Fibrosarcoma is the prototypical soft tissue tumor: it may be recognized in virtue of its peculiar "herring bone" pattern due to haphazardly arranged fascicles of spindle cells. If compared to SS, fibrosarcoma is more disorderly with cellular fascicles intersecting at haphazard angles; vortoicoid ("neuroid") whorling is unusual in fibrosarcoma. On the other hand, the nuclear atypia as well as brisk mitotic activity encountered in fibrosarcoma are less commonly seen in SS. Distinction of SS from malignant peripheral nerve sheath tumors (MPNST) may pose irreducible diagnostic difficulties on a pure histologic ground since both the conditions share some architectural, cytologic as well as immunochemical features. However, MPNST often occurs in the background of neurofibromatosis; the anatomic relationship to a nerve trunk or a clinically pre-existent neurofibroma can be often demonstrated. Microscopically, fascicles of MPNST differ from those of SS having less lobulation at the periphery; tumor cells are larger and, usually, and have wavy or bullet shaped nuclei as well as more atypical chromatin texture. Ground substance collagenization, calcifications or ossifications, common in SS, are generally lacking in MPNST. Leiomyosarcoma is distinctly made up of tumor fascicles intersecting at right angle, a pattern which is easy to catch in resection specimen but can be overlooked in core biopsies. Tumor cells have slender nuclei with blunt-end nuclei and stainable cytoplasm with fibrillary qualities. Paranuclear vacuoles may be present. Immunohistochemical demonstration of desmin is a reliable indicator of muscle differentiation.

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QUESTIONS

1. Which of the following is true in regards of synovial sarcoma:
   - **A.** Most lesions occurs in the elderly
   - **B.** S100 protein is a reliable diagnostic means
   - **C.** Prognosis may be related to size
   - **D.** Calcification is uncommon
   - **E.** Distinction from other sarcomas is easy
2. The differential diagnosis of synovial sarcoma may include:
   - **A.** Metastatic sarcomatoid squamous carcinoma
   - **B.** Leiomyosarcoma
   - **C.** Malignant schwannoma
   - **D.** Fibrosarcoma
   - **E.** All of them

***Mark the <u>best</u> answer, not just a good answer!**

1. Quale delle seguenti affermazioni –riferita al sinovialsarcoma- è vera.
   - **A.** La maggior parte delle lesioni si osserva negli anziani.
   - **B.** La Proteina S100 è un attendibile marker diagnostico.
   - **C.** La prognosi è correlata alla dimensione.
   - **D.** Le calcificazioni sono rare.
   - **E.** La distinzione dagli altri sarcomi è facile.
2. La diagnosi differenziale del sinovialsarcoma può includere:
   - **A.** Metastasi di carcinoma squamoso sarcomatoide.
   - **B.** Leiomiosarcoma
   - **C.** Schwannoma maligno.

D. Fibrosarcoma.
E. Tutte le entità sopra enunciate.

***Contrassegnare la migliore risposta, non già solo una buona risposta!**

Case 20. 2761-00

Janez Lamovec, M.D., Department of Pathology, Institute of Oncology, Ljubljana, Slovenia.

CLINICAL HISTORY

A 59-year-old woman was admitted to the hospital because of a recently enlarging goiter that she has had for 15 years. Ultrasonographically, an enlarged hipoechogenic non-homogeneous right lobe of the thyroid was found. By FNAB anaplastic carcinoma was diagnosed. The tumor was considered to be inoperable. The patient was irradiated and received chemotherapy treatment with Adriablastin. Tumor regressed appreciably following such a treatment (from 10 cm in its largest dimension to 5 cm) and was deemed to become operable. An extracapsular right lobectomy was performed.

PATHOLOGY

Grossly, the specimen was represented by a right lobe of the thyroid which measured 7 x 7 x 4.5 cm and weighed 90 grs. On cut surface, the lobe was completely replaced by confluent multinodular necrotic and hemorrhagic tumor of variegated color and soft and friable in consistency. It was surrounded by an unevenly thick capsule which was not overgrown by tumor.

Microscopically, the nodule(s) is largely necrotic, with fibrin and blood admixed with necrotic material. Peripherally to necrotic areas, a meshwork of anastomosing highly irregular vascular structures are seen set in a fibrosed stroma. In addition, trabeculae and ribbons of neoplastic cell without clear lumina are present in some foci, and also a few almost solid foci of such cells are seen. In some areas neoplastic cells line irregular dilated spaces, partly filled with blood. Some of such spaces are empty, surrounded by thick fibrinous homogeneous material in which neoplastic cells form dense retiform structures with papillary protrusions lined by neoplastic cells.

Neoplastic cells are predominantly epithelioid, with basophilic cytoplasm and large clear nuclei with very distinctive large, occasionally elongated nucleoli. Mitoses are numerous. In some tumor cells, intracytoplasmic lumina are seen.

Tissue surrounding the tumor nodule(s) is fibrotic, with inflammatory cells, deposits of hemosiderin and relatively sparse preexistent thyroid follicles, and in just a few foci there are dense groups of microfollicles suggesting remnants of follicular adenoma. In some areas, neoplastic infiltrate and fibrosis involve skeletal muscle fibres on the surface of the specimen.

Immunohistochemically, most tumor cells were strongly positive for CD31 and CKMNF116, most or many cells were also positive for Factor 8 and CD34. They were negative for thyroglobulin in contrast to follicular epithelium which was positive; the latter also showed positivity for CKMNF116. Rare neoplastic cells were also weakly positive for EMA; follicular epithelium was also weakly and unevenly positive for the latter marker.

DIAGNOSIS

Epithelioid angiosarcoma of the thyroid (? arising in a follicular adenoma).

FOLLOW-UP

The patient was postoperatively irradiated and received chemotherapy. Six months following thyrodectomy, she had a pathological fracture of the right clavicula and a few months later multiple lung metastases and pleural effusion appeared. She died one year after thyreodectomy with disseminated disease. No autopsy was performed.

COMMENT

The discussion on angiosarcoma of the thyroid has been traditionally centered on the question of its very existence. One group of authors think that most of tumors showing angiomatoid appearance are true angiosarcomas of endothelial origin (1-2) while others believe that such tumors represent anaplastic thyroid carcinomas simulating angiosarcoma or poorly differentiated epithelial malignancy with a capacity for divergent endothelial differentiation (3-4). By the latter group of authors, the demonstration of immunoreactivity for Factor 8, CD 31 or CD34 is not enough evidence for endothelial derivation of the tumor. Factor 8 may be nonspecifically absorbed from the blood by anaplastic carcinoma cells - and anaplastic carcinomas of the thyroid are highly vascularized and hemorrhagic tumors – while CD31 and CD34 may be expressed by a minority of thyroid anaplastic carcinoma (4). On the other hand, the unequivocal ultrastructural demonstration of endothelial differentiation of tumor cells, such as documentation of cytoplasmic Weibel-Palade bodies and some other ultrastructural features may be considered as a definite proof for a bona fide entity of angiosarcoma (5-6). Furthermore, it was shown recently by in situ hibridization technique that all studied cases of angiosarcoma of the thyroid failed to express thyroglobulin mRNA while all cases of anaplastic carcinomas at least weakly gave a positive signal (7).

The presented case is an example of epithelioid variant of angiosarcoma. As expected, such tumors, in addition to positive vascular markers also express epithelial markers, such as keratin. This was observed before, in epithelioid angiosarcomas of thyroid (6), soft tissues (8,9), etc. It appears that tumors that look epithelioid on H&E may also be such immunohistochemically. Furthermore, basic studies on the nature of endothelial cells suggested particular relationship between certain subsets of endothelial and epithelial cells (10).

It is known that angiosarcoma of the thyroid, extremely rare tumor, was most commonly reported from Alpine countries (2, 11) although reports from non-Alpine regions are also on record (12, 13). Slovenia is in a large part an Alpine country and as such belong to the "thyroid angiosarcoma belt" and, indeed, angiosarcomas of the thyroid from this region have been previously reported (14). Tumors most commonly arise in the setting of longstanding nodular goiter (a common disease in those countries); in our case the tumor probably arose inside an uninodular goiter or follicular adenoma.

In differential diagnosis, practically the only tumor to be considered is anaplastic thyroid carcinoma, particularly its angiomatoid variant (3-4). In addition, at least hypothetically, some peculiar vascular proliferations in the thyroid, such as extravascular and intravascular papillary endothelium hyperplasia (15-16) may mimic low grade angiosarcoma.

Angiosarcoma of the thyroid is a high grade sarcoma with dismal prognosis and as such only theoretically important to be distinguished from equally fatal and more common anaplastic thyroid carcinoma.

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QUESTIONS

1. Angiosarcoma of thyroid is most commonly associated with:
   - **A.** Hashimoto thyroiditis.
   - **B.** Papillary carcinoma.
   - **C.** Preexisting hemangioma.
   - **D.** Long-standing nodular goiter.
2. Epithelioid angiosarcoma is often immunohistochemically positive for keratins.
   - **A.** True.
   - **B.** False.

***Mark the appropriate letter.**

1. L'angiosarcoma della tiroide è molto spesso associato a:
   - **A.** Tiroidite di Hashimoto.
   - **B.** Carcinoma papillare.
   - **C.** Emangioma pre-esistente.
   - **D.** Gozzo nodulare di lunga data.
2. L'angiosarcoma epitelioide è spesso immunoistochimicamente positivo per citocheratine.
   - **A.** Vero.
   - **B.** Falso.

***Contrassegnare la lettera giusta.**

CASE 21. M31797/01

Michal Michal, M.D., Sikl's Department of Pathology, Charles University, Medical Faculty Hospital, Pilsen, Czech Republic.

CLINICAL HISTORY & PATHOLOGY

60 year-old woman with a tumor, which was described by clinician to arise in the dermis of the submandibular region. The tumor was 2 cm in size. It had white color, hard consistency. Immunohistochemically the tumor cells were strongly S-100 protein and cytokeratin positive.

DIAGNOSIS

Clear cell malignant myoepithelioma of the submandibular gland.

COMMENT

Non-neoplastic myoepithelial cells may be spindle-shaped, plasmacytoid (hyaline), or clear (7) Likewise, the cells that constitute benign myoepithelioma may also display this range of appearances, although most lesions are composed of either the spindle or plasmacytoid types (8). Sometimes, these tumors (and the closely related pleomorphic adenoma) also contain a minor component of clear cells, but it is only occasionally that these cells are the majority type. For example, in the 40 benign myoepitheliomas described by Dardick et al (1), only one had a predominance of clear cells. Two similar tumors were reported recently, one each occurring in the parotid and minor salivary glands. Both were well circumscribed, lacked necrosis, and were composed of extensive sheets of regular clear cells. One of them included a minor component of spindle-shaped cells. Both tumors expressed S-100 protein and the parotid lesion showed patchy positivity with a smooth muscle actin (2).

Malignant myoepitheliomas are much less common than their benign counterparts (9). The criteria for diagnosing malignancy in a myoepithelial neoplasm was said to include necrosis, cytological pleomorphism, increased mitotic activity and, particularly, an invasive growth pattern. The neoplastic cells had several different appearances, including spindle-shaped, stellate, plasmacytoid, or epithelioid types and, in addition, two tumors displayed foci of squamous metaplasia. Amongst other reports of malignant myoepithelioma (10,11,12,13,14,15,16,17,18,19), only three (20,21,22) alluded to clear cell change as a minor component. Indeed, the only definite identifiable example of a clear cell malignant myoepithelioma is case 1 in the series of monomorphic clear cell neoplasms of Ogawa et al (3). This was a palate tumor that recurred 3 years after excision. It was composed of nests and cords of polyhedral cells with abundant clear cytoplasm. There was mild cellular and nuclear pleomorphism, and extensive areas of necrosis were present. Most tumor cells expressed S-100 protein and vimentin with foci of cytokeratin positivity and, also, electron microscopy supported a myoepithelial origin. In addition, two other probable examples, in the palate (23) and submandibular gland (24) respectively, were each described as a monomorphic clear cell carcinoma arising in a pleomorphic adenoma, and both showed immunohistochemical evidence of myoepithelial differentiation.

We have recently published several cases of clear cell malignant myoepitheliomas (25). Two of the five published cases were originally thought to be a primary, clear cell adnexal tumor of the skin. When the tumors were thoroughly sampled, rests of normal salivary glands were encountered. In the study (25), each of the tumors was a malignancy composed of a single population of optically clear cells, in addition to which Case 4 possessed a minor component of spindle-shaped cells. All five neoplasms showed

immunohistochemical evidence of myoepithelial differentiation by expressing S-100 protein and, to a lesser extent, a smooth muscle actin. Furthermore, all tumors displayed collagenous spherules - these are round, eosinophilic bodies up to 100 mm in diameter comprising mucin, elastin, basement membrane proteins such as laminin and type IV collagen, admixed with collagen types I and III (25). They are produced by neoplasms and tumor-like lesions of myoepithelial origin in the salivary glands (26), and also in the breast (6,27) and in dermal chondroid syringomas (28). These collagen spherules were not well seen in the two blocks cut for this seminar. They were, however, seen in other block of the same neoplasm.

The differential diagnosis of clear cell malignant myoepithelioma encompasses all other tumors that may be composed of clear cells. However, the closest similarity is to the epithelial form of monomorphic clear cell carcinoma, which is also an invasive malignancy composed of a single population of clear cells, and which often displays stromal hyalinization. These neoplasms express cytokeratin, but are always negative with S-100 protein and a smooth muscle actin, unlike those in the present study. The absence of a significant epithelial component differentiates our tumors from epithelial-myoepithelial carcinoma. However, it is now apparent that the latter has a wider morphological range than previously thought, and it could be conceivable that there may be an overlap with some forms.

Clear cell malignant myoepitheliomas are usually neoplasms of low grade malignancy. Only one patient, Case 4 suffered a recurrence (in fact five times), and she now has clinical evidence of lung secondaries (25). The tumor of Ogawa et al (3) recurred after 3 years, and that reported by Cassidy & Connolly (24) metastasized and caused death after 20 months. In contrast, the patient of Klijanienko et al (23) was disease free after a year.

Clear cell variant of malignant myoepithelioma needs to be separated from other clear cell neoplasms of the salivary glands, in particular, hyalinizing clear cell carcinoma (4,5).

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QUESTIONS

1. Can collagenous spherulosis, which is a typical structure seen in clear cell myoepithelial carcinoma of the salivary glands, be seen in tumors other than the salivary glands? **Yes or No.** ____.
2. Is the clear cell myoepithelial carcinoma of the salivary glands related to epithelial-myoepithelial carcinoma of the salivary glands? **Yes or No.** ____.

1. La sferulosi collagenica, che è una struttura che si osserva tipicamente nel carcinoma mioepiteliale delle ghiandole salivari, può osservarsi su tumori extra-salivari? **Si o No.** ____.
2. Il carcinoma a cellule chiare mioepiteliali delle ghiandole salivari è correlato al carcinoma epi-mioepiteliale delle stesse ghiandole salivari? **Si o No.** ____.

CASE 22. 1000334-99

Michele Bisceglia, M.D., Department of Pathology , IRCCS-Hospital "Casa Sollievo della Sofferenza", San Giovanni Rotondo (FG), Italy

CLINICAL HISTORY

A 57-year old woman of Albanian origin with a left facial nerve paresis of 5 year duration associated with frequent ipsilateral attacks of headache. Recent episode of loss of consciousness. At admission a sensorineurial hearing loss as well as cerebellar ataxia were also recorded. At CT and MRI scans a large lytic destructive tumor with intense contrast enhancing was documented involving the temporal bone with extension into the posterior cranial fossa and invasion of the adjacent cerebello-pontine angle. Angiography displayed a persistent characteristic tumoral blush as well as the involvement of the sigmoid sinus and jugular bulb, the latter evidence being confirmed intraoperatively. On a presumed preoperative diagnosis of aggressive meningioma a partial tumor resection limited to the intracranial portion was performed

PATHOLOGICAL FINDINGS

Histological appearances

The tumor appears as a papillary and focally cystic proliferation lined by a single row of cells ranging from a flattened or attenuated in appearance to columnar with uniform nuclei situated either in the center of the cells or toward the luminal aspect, and with pale eosinophilic to clear appearing cytoplasm. Underlying or adjacent to the epithelial proliferation one can see also an intense inflammatory tissue reaction with cholesterol granulomas, hemosiderin deposits, and fresh hemorrhages. Some cases may have hypercellular areas with crowded cystic glands having a distinct thyroid-like appearance and containing eosinophilic colloid-like material. Some other cases still are characterized by areas of hypocellularity with a marked fibrosing stromal response.

Immunohistochemistry

The tumor, which is usually cytokeratin positive, may also exhibit positivity for vimentin and neuroectodermal markers such as S-100 protein, glial fibrillary acidic protein, synaptophysin, neuron-specific-enolase, and Leu-7.

DIAGNOSIS

Endolymphatic sac papillary tumor –Heffner tumor.

DISCUSSION AND COMMENTS

Endolymphatic sac papillary tumor also currently known under the eponymic term of Heffner tumor is a rare neoplasm of which –including all tumors variously designated- less than 70 cases were reported up to 1996 (1). Based on a computerized bibliography search to date around further 63 cases have been since 1996 so far reported making a grand total of 133 cases (2). Various –appropriate as well as inappropriate- labelling terms have been used in the literature for describing this type of lesion such as (papillary) adenoma of the endolymphatic sac, adenoma/adenocarcinoma of middle ear/temporal bone, papillary adenoma of temporal bone, aggressive papillary middle ear/temporal bone tumor, adenoma/adenocarcinoma of the ceruminous gland, low grade adenocarcinoma of probable endolymphatic sac origin, (aggressive) (papillary) tumor/neoplasm of the endolymphatic sac, Heffner tumor (3-4), and (clearly inappropriately) (heterotopic or primary) choroid plexus papilloma of the cerebello-pontine angle, this reflecting uncertainty regarding the

nature, histogenesis, and biological behaviour of the tumor. Obviously the erroneous diagnosis of the endolymphatic sac tumor under specific and mostly confusing terms referring to middle ear gland neoplasms does not imply that all middle ear gland neoplasms are endolymphatic sac tumors.

The origin of the lesion is probably still a matter of debate. In addition to a choroid plexus heterotopia based-hypothesis favoured by some authors for these papillary tumors, which is currently abandoned, and to a middle ear mucosa-derived entodermal histogenesis (5), actually topographical and anatomical (histological, immunohistochemical and ultrastructural) data mainly due to a tentative study of a large series of cases by Heffner (6) correlated with the clinicopathological features of the lesion are in support of an endolymphatic sac origin and neuroectodermal histogenesis (1,6,7,8). Among these data we mainly count the location of tumor

(i.e. the region of the posterior/medial petrous ridge, as the site of the normal endolymphatic sac), the resemblance of the normal endolymphatic sac epithelium to the tumor itself, and the immunohistochemical results (cf above). Accordingly we consider the endolymphatic sac tumor as a separate entity distinct from the true entodermally derived tumors of the middle ear and temporal bone (pneumatic air spaces), which in turn can possibly exhibit either pure mucinous (adenomatous) or neuroendocrine (carcinoid) or combined (amphicrine) histochemical and ultrastructural differentiation (9). Although the endolymphatic sac tumor was initially considered to be a low-grade adenocarcinoma by the patronimic author (6), the thought of most authors including Heffner himself is currently that these tumors are benign with locally infiltrative growth but no metastatic capabilities (1,3).

The tumor has a slight female sex predilection and occurs over a wide age range from the 2^{nd}-8^{th} decades of life. The most common symptoms include vertigo and unilateral hearing loss. The hearing loss is most frequently sensorineural rather than conductive, but mixed types of hearing loss also occur. Other symptoms include tinnitus, ataxia and other lower cranial nerve palsies. The etiology is unknown but consistent clinical evidence deriving from old (10) as well as recent literature has shown that this lesion may be part (in around 10-15 per cent of cases) (1,5,11) of von Hippel-Lindau syndrome (VHL) either of type 1 syndrome (without pheochromocytoma) or type 2 (with pheochromocytoma). VHL syndrome is an autosomal dominant, multisystem neoplastic disorder genetically linked with a germline mutation of a tumor suppressor gene (VHL gene), located on chromosome 3p25 (12), in which tumor development is due to inactivation or loss of the remaining wild-type allele in susceptible cells of various organs. Todate there are also available molecular methods of investigation (13-14) as well as fluorescence in situ hybridization tests allowing genetic analysis for diagnostic testing, which have been also in part performed on tumor cells derived from the endolymphatic sac tumor (15-16), with results in support of this lesion as one of the visceral stigmata of VHL. Parenthetically the first patient described by Eugen von Hippel was affected by a temporal bone tumor, probably corresponding to endolymphatic sac tumor (17). Endolymphatic sac tumors may also occur bilaterally (18), more often in the context of VHL syndrome (11).

Thus the endolymphatic sac tumor should be included in the list of the possible phenotypic expressions characterizing this clinical condition, so enlarging the spectrum of those less common but numerous other manifestations of the syndrome (such as syringobulbia, syringomyelia, renal angioma, splenic angioma, splenic cyst, hepatic cyst, hepatic adenoma), in addition to the more common or classical manifestations, such as retinal or central nervous system hemangioblastomas, renal cell carcinoma, pancreatic cysts/ /cystadenoma/ /carcinoma/ /islet cell tumor, adrenal pheocromocytoma, epididimal papillary cystadenoma in men, and pelvic tumor of the broad ligament of wolffian origin in

women, all of them characterized by a highly rich vasculature by virtue of the main effect of the gene product (pVHL) which maily regulates angiogenesis (13-14). Mutation of the VHL tumor suppressor gene has been documented not only in endolymphatic sac tumor patients with a familial history, but also in sporadic cases (16). As a clinical consequence it should be recommended to screen all patients diagnosed with endolymphatic sac tumors for the other manifestations of the VHL syndrome as well as conversely to screen all patients with VHL syndrome for the detection of the endolymphatic sac tumor or for a simple hearing loss even in the absence of radiographic evidence of an endolymphatic sac tumor (11).

DIFFERENTIAL DIAGNOSIS

RADIOLOGICAL DIFFERENTIAL DIAGNOSIS

The radiological features of endolymphatic sac tumor, mostly CT scan and MRI investigation, usually reveal a contrast-enhancing lytic temporal bone lesion measuring 4-6 cm, with the typical location for this lesion seen at or near the posterior-medial face of the petrous bone (19-21) . The tumor may extend to the middle and posterior cranial cavity, resulting in cerebellar involvement or/and compression and/or shifting of the 4th ventricle, brain stem or pineal gland. Angiographic studies constantly show a vascular or hypervascular lesion which usually suggest a preoperative diagnosis of a vascular-like tumor, i.e. first a jugulo-tympanic paraganglioma or angioblastic meningioma.

The radiological differential diagnosis should consider inflammatory processes (middle ear or mastoid cholesteatoma, cholesterol granuloma, Wegener's granulomatosis, tuberculoma, non-tuberculous granulomatous inflammation) as well as neoplastic lesions (dermoid-epidermoid cyst, acoustic neuroma, schwannoma, intrinsic jugulo-tympanic paraganglioma, primary middle ear ceruminoma//adenoma//adenocarcinoma, carcinoid tumor of the middle ear, hematolymphoid neoplasm, primary squamous cell carcinomas, adjacent infiltrating or ectopic meningioma, ectopic pituitary adenoma, enterogenous or arachnoidal cysts, infiltrating glioma, and metastatic seeding from unknown primary). Exceptional lesions of miscellaneous etiology should also be taken into account such as some of vascular (internal carotid artery aneurism, arterovenous malformation, and others) or of disreactive origin (amyloidotic pseudotumor).

HISTOLOGICAL DIFFERENTIAL DIAGNOSIS

Analogously the histological differential diagnosis includes reactive as well as neoplastic conditions. Inflammatory processes with granulation tissue and prominent stromal response can either result in overdiagnosis (chronic mastoiditis –Fig. 4 Ref. 1) or in overlooking the correct diagnosis if only limited areas of tumor are preserved. Papillo-tubulo-cystic primaries, either intrinsic or from adjacent structures, including middle ear adenoma/adenocarcinoma (3, 9, 22-23), ceruminal gland adenoma/adenocarcinoma (24-25), salivary choristoma (26), carcinoid tumors of the middle ears (27-28), choroid plexus papilloma (29), meningiomas of the usual (30a) as well as papillary type (31), and metastatic adenocarcinoma (30b) -particularly papillary carcinoma of the thyroid, renal cell carcinoma, bronchogenic carcinoma- can mimick or masquerade the endolymphatic sac tumor.

BIOLOGIC BEHAVIOUR

Despite being slowly-growing, with symptoms or radiologically-based findings dating back even to 20 years, this neoplasm is capable of widespread infiltration and destruction and may be lethal as a result of extension into vital structures with the development of meningitis. For many years, although included in the group of the middle ear and mastoid adenomatous benign tumors (4,32-33), this neoplasm has been credited of a more

aggressive behaviour, the morphological marker of which had been recognized in the papillary architecture as opposed to the the mixed architecture which seemed more consonant with the behavior of truer adenomas and carcinoids (3).
Thus, the treatment of choice is radical surgery, including mastoidectomy and temporal bone resection which may necessitate the sacrifice of cranial nerves. Radical surgery is potentially curative. An inadeguate surgical removal would be followed by local recurrence with subsequent much higher operative morbidity. Prognosis is dependent on the extent of disease and the adequacy of resection, with small and confined tumors –if early detected- surgically cured with low operative associated morbidity.

FOLLOW-UP

Following the diagnosis of endolymphatic sac papillary tumor, this case was systematically studied by physical examination and imaging investigations and the patient found free of any stigmata of the von Hippel-Lindau syndrome (Heffner tumor –sporadic type). A radical surgical intervention of petrousectomy was planned, which the patient refused.

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QUESTIONS

1. A. Is Heffner tumor eponymic with endolymphatic sac papillary tumor? **Yes or No. ____.**
   B. Is aggressive papillary adenoma of the middle ear/temporal bone tumor synonymic with endolymphatic sac papillary tumor? **Yes or No. ____.**
2. A. Does Heffner tumor have local aggressiveness? **Yes or No. ____.**
   B. Has this tumor metastasizing capability? **Yes or No. ____.**

1. A. L'etichetta di tumore di Heffner è eponimica del tumore papillare del sacco endolinfatico? **Si o No.** ____.
   B. Il termine di adenoma papillare aggressivo dell'orecchio medio/osso temporale è uno dei sinonimi del tumore papillare del sacco endolinfatico? **Si o No.** ____.
2. A. Il tumore di Heffner ha comportamento locale aggressivo? **Si o No.** ____.
   B. Ha questo tumore capacità di metastatizzare? **Si o No.** ____.

SURGICAL PATHOLOGY -SESSION VI. HEMATOLYMPHOID TISSUE

CASE PRESENTATIONS (5 cases)

Case 23. Cerebral intravascular large B-cell lymphoma

(*J. Forteza-Vila*)

Case 24. MALT lymphoma of the small bowel

(*S. Ramon y Cajal*)

Case 25. BCL-2 negative non-Hodgkin follicular lymphoma

(*J. Forteza-Vila*)

Case 26. Subcutaneous, panniculitis-like T-cell lymphoma

(*N. Weidner*)

CASE 23. (A)01-0038-32 or 01-0038

Jerónimo Forteza-Vila, M.D., School of Medicine and University Clinical Hospital in Santiago de Compostela.

B. Vieites, M. Fraga, J. Forteza
School of Medicine and University Clinical Hospital in Santiago de Compostela.

CASE DESCRIPTION

CLINICAL HISTORY

Male, 73 years old, diabetic, who enters the hospital after a sudden episode of deviation to the left of the buccal commissure and loss of strength in right extremities. The CAT (computerized axial tomography scanner) showed right cerebellar hypodensity. The patient was discharged with the diagnosis of lacunar infarction.

After a month, he comes again due to holocraneal cephalea, uneven running, difficulty to concentrate and loss of memory. The radiological study shows ischaemic lesions predominantly subcortical in the semioval centre, right parieto-occipital area and the right part of the rounding of the corpus callosum. Considering the symptomatology and the radiological findings, the possibilities of *vasculitis* or *primary lymphoma of the C.N.S.*

The patient was moved to our centre in order to complete the study. During his stay, he experienced light clinical oscillations with fever peaks, alteration of the level of consciousness and a progressive tendency to sleep and the radiological tests show ischaemic images which are similar to the previous ones. (MNR). The patient died four months after the beginning of the symptoms with a progressive worsening of his general state.

AUTOPSY

An autopsy was carried out and it confirmed the ischaemic lesions observed in the MNR. The histological study showed the existence of a proliferation of atypical lymphoid cells, located into the lumen of small vessels of the CNS, lungs, heart, adrenal fat, bone marrow and spleen. The tumor cells have a large size with multiple prominent nucleoli and abundant mitosis. The immunopehotype showed positivity for CD20, bcl2 and a high proliferative rate (MIB1). The study by means of PCR proved the existence of translocation t(14:18), which showed a possible follicular origin for the tumor.

The patient also presented signs of sepsis (hemoculture: Klebsiella pneumoniae) and myocardial ischemia which contributed to the final exitus.

DIAGNOSIS

Intravascular large B-cell lymphoma.

DISCUSSION

The intravascular lymphoma is an uncommon variety of highly aggressive non-Hodgkin Lymphoma, in which tumor cells are located in the lumen of small vessels, obstructing them. In the literature around 200 cases have been described; most of them corresponding to a LNH of B strain, though there are published cases of intravascular T-cell LNH.

The exclusively intravascular location of the neoplastic cells has been explained as a consequence of the absence of cell-surface receptors known as "homing". There are studies proving that the negativity of the labelling with monoclonal antibodies for adhesion molecules such as CD29 (beta 1 integrin) and CD54 (ICAM-1), responsible for the transvascular migration of lymphocytes.

The immunophenotype of these tumors is variable, and being mostly B-cell neoplasias for CD10, CD5 and cyclin D1. Its origin has been discussed many times and, as far as now, it could have not been proved. In this case, we have carried out a molecular study with the tissue in paraffin, and we obtained the translocation t(14:18) in several occasions, which makes us think about the possibility of a centrofollicular origin in some of these neoplasias.

The intravascular lymphomas are highly aggressive, with an average survival of 5 months and a post-mortem diagnosis in most cases. This is due to the difficulty in the clinical diagnosis because of the heterogeneity of the symptoms and to the fact that biopsies are rarely performed. However, there is evidence of a response to combined chemotherapy treatment, with remission rates up to the 43%.

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QUESTIONS

1. The intravascular lymphoma is:
   - **A.** A vasculitis.
   - **B.** A lymphoma MALT- type.
   - **C.** Generally a T- lymphoma.
   - **D.** a leukemia lymphoma.
   - **E.** The same as the intravascular angioendoteliomatosis described in the skin.
2. The intravascular lymphoma in the brain gives clinically:
   - **A.** Mass effect
   - **B.** Dementia
   - **C.** Temporary ischaemia
   - **D.** Leukemic manifestation
   - **E.** A "vasculitis-like" syndrome

***Mark the appropriate letter.**

1. Il linfoma intravasculare è:
   - **A.** Una vasculite.
   - **B.** Un linfoma MALT-type.
   - **C.** In genere un linfoma-T.
   - **D.** Un linfoma leucemizzato.

   E. La stessa entità descritta nella cute come angioendoteliomatosi intravascolare.

2. Il linfoma intravascolare nel cervello dà clinicamente:
   A. Effetto massa.
   B. Demenza.
   C. Ischemia transitoria.
   D. Manifestatione leucemica.
   E. Una sindrome "vasculitis-like"

***Contrassegnare la lettera giusta.**

CASE 24. 954212

Santiago Ramon y Cajal, M.D., Department of Pathology, Clinica Puerta de Hierro, Madrid, Spain

CLINICAL HISTORY

A 17-year-old female with moderate pancytopenia after vaccination against B Hepatitis. A bone marrow biopsy was performed and an infiltration by a Low Grade Lymphoproliferative Process was suggested. After an exhaustive study, a 4.5 cm jejunal tumoral lesion was resected. Splenectomy was considered and, finally, performed. Representative slides from jejunal lesions are submitted for seminary purposes.

PATHOLOGIC FINDINGS

The bone marrow biopsy showed a hypocellular marrow and some areas with a very loose infiltrate of mononuclear cells, which appeared lymphoid, but without significant atypia.
Grossly, a surgical specimen showed a mesenteric mass in close relation to a 16.5 cm segment of small bowel. The tumor ulcerated the mucosa of the bowel and the cut surface was white. A mesenteric lymphadenopathy was also obtained. The mass showed diffuse proliferation affecting mucosa, muscularis propria and serosa of small and medium size lymphocytes with round or irregular nuclei, with sparse plasma cells.

Mesenteric lymph node showed a partially altered structure, with variable and irregular follicles and a polymorphous lymphoid population. Small lymphocytes with abundant cytoplasms predominated, and they invaded the capsule of the lymph node and mesenteric adipose tissue.

Immunohistochemically, B markers (L26, CD19) were positive and an accompanying (and in some fields predominant) T cell population (CD3, UCHL-1 and CD45Ro positive) was detected in the mesenteric lymphadenopathy. The TRAP technique showed some histiocyte-like cells. Bcl-2 was positive in some cells inside the germinal centers. CD5, CD10, CD23, CD30 and EBV were negative. A low percentage of MIB2 was detected. Heavy chains of immunoglobulin rearrangements were diagnosed by PCR.

Flow cytometry detected the following cellular populations: CD3 + CD4 = 38%; CD3 + CD8 = 29%; CD3 + CD25 = 0.9%; CD3 + Dr = 6.2%; CD19 + Kappa = 5.5%; CD19 + Lambda = 9.1%; CD20 + Kappa = 3.8%; CD20 + Lambda = 9.7%; CD103 = 1.9%

H. Pylori was demonstrated after surgery, in a gastric biopsy.

DIAGNOSIS

Low Grade Lymphoproliferative Process, probably a Low-grade lymphoma of mucosa-associated lymphoid tissue (MALT).

DISCUSSION

Other pathologists were consulted and reported several diagnoses, including centrofollicular lymphoma, peripheral T lymphoma, lobulated small and intermediate cell lymphoma, a highly abnormal antigen-driven immune response still responsive to host immune surveillance, and High Grade Large B cell Lymphoma-T cell rich variant.
We submit this case because it was interpreted differently by expert hematopathologists,

and some of these had contributed to establishing the REAL Classification of Lymphomas. This example highlights the difficulties in classifying lymphomas and diagnosing unusual cases.

Case 24. QUESTIONS for CME assessment

1. In a bone marrow biopsy with sparse lymphoid cells, in a 17 year old woman, you should think in:
   - **A.** Low grade lymphoproliferative process.
   - **B.** A reactive inflammatory or autoimmune process.
   - **C.** A malignant lymphoma.
   - **D.** Hairy cell leukemia.
   - **E.** All diagnoses are possible.

   ***Mark the appropriate letter.**
2. MALT lymphomas always are diagnosed in their primary location?
   Yes or No. ____.

1. La presenza di sparse cellule linfoidi in una biopsia osteomidollare, in una giovane donna, farebbe pensare a:
   - **A.** Un processo linfoproliferativo di basso grado.
   - **B.** Un processo infiammatorio o autoimmune.
   - **C.** Un linfoma maligno.
   - **D.** Una leucemia a cellule capellute.
   - **E.** Tutte le possibilità sopra elencate.

   ***Contrassegnare la lettera giusta.**

2. I linfomi MALT sono sempre diagnosticati nella loro sede primitiva?
   Si o No. ____.

CASO 25. 1825-99 or 3349-01

Jerónimo Forteza-Vila, M.D., School of Medicine and University Clinical Hospital in Santiago de Compostela.

B. Vieites, M. Fraga, J. Forteza
School of Medicine and University Clinical Hospital in Santiago de Compostela.

CLINICAL CASE:

Male aged 34, with a left lateral facial tumor which corresponded to parotid tissue and lymph node macroscopically. There were lymph adenopathies in two areas, cervical and axillary. These adenopathies had several years of evolution. HIV serology was negative.

FIRST BIOPSY

Histologically, follicles with typical reactive follicular hyperplasia were observed. Reactive germinal centers with plenty of macrophages with Fleming bodies were observed. These germinal centers were CD20 positive Bcl2 negative.

In other fields, the germinal centers had the same size diameters and they tended to coalesce. On a focal character, sclerosis between the follicles was observed.

We have found that the IgH rearrangement in paraffin tissue was polyclonal, but repeatedly translocation (14:18) was found in the mbr point.

SECOND BIOPSY

A second biopsy of a left axillary adenopathy which had diameters of 6 x 4 was performed approximately after a year and the results of immunohistochemistry and of the M.B. as the morphology were identical.

Peripheral blood, bone marrow aspirate and biopsy were uninvolved from a morphological and molecular point of view, and therefore, the decision of "wait and see" was taken. In any case, in this second biopsy the monotony of some follicles Bcl2 negative was more evident and they frequently showed a reduced mantle zone, almost disappeared with the IgD immunostaining. Some of these follicles had a diminished proliferation rate consistent with a neoplastic nature.

The Bcl6 staining showed that there were germinal center cells in interfollicular spaces.

A subsequent bone marrow biopsy showed infiltration by lymphoma (CD20 positive) and, although this finding was discordant with the morphological evaluation of bone marrow aspirate (free of disease), it was in accordance with the emergence of a t(14:18) in the aspirate, identical to that found in the lymph node.

The patient's evolution in two years and a half is asymptomatic with bilateral axillary adenopathies of changing size.

DIAGNOSIS

BCL2 NEGATIVE FOLLICULAR LYMPHOMA. The diagnosis of these cases require a careful clinico-pathological and molecular study. The dissociation between M.B. and immunophenotype could be explained by several hypothesis:

a) From a phenotypical standpoint, bcl2 epitopes could be altered in an abnormal protein and could not be detected with conventional antibodies.

b) From a genotypical point of view, there could be either a mutation of the IgH promotor or the bcl2 gene that could explain the absence of protein.

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QUESTIONS

1. From an immunohistochemical point of view, the follicular lymphoma is always:
   - **A.** CD20 positive.
   - **B.** BCL2 positive.
   - **C.** Translocation 14:18 positive.
   - **D.** CD5 positive.
   - **E.** CD3 positive.
2. The follicular lymphoma is marked cytogenetically:
   - **A.** By the translocation 14:18.
   - **B.** By the translocation 4:14.
   - **C.** By aneuploidy
   - **D.** By trisomia 21
   - **E.** There is no translocation.

***Mark the appropriate letter.**

1. Da un punto di vista immunoistochimico il linfoma follicolare è sempre:
   - **A.** CD20 positivo.
   - **B.** BCL2 positivo.
   - **C.** Traslocazione 14:18 positivo.
   - **D.** CD5 positivo.
   - **E.** CD3 positivo.
2. Il linfoma follicolare è caratterizzato citogeneticamente da:
   - **A.** Traslocazione 14:18.
   - **B.** Traslocazione 4:14.
   - **C.** Aneuploidia
   - **D.** Trisomia 21.
   - **E.** Nessuna traslocazione.

***Contrassegnare la lettera giusta.**

Case 26. (S)01-2507

Noel Weidner, M,D. Department of Pathology, University of California, San Diego Medical Center, San Diego, CA, USA

CLINICAL HISTORY

A 56 y/o Filipino female presented with a 4-month H/O acute onset of multiple, painless, subcutaneous nodules of varying sizes. Following diagnosis (3/14/01), the patient was started on polyagent chemotherapy. She received 10 weeks of a12 week regimen, when she presented with severe pulmonary symptoms. Bronchial washings showed filarial larval parasites c/w *Strongyloides stercoralis* (Strongyloidiasis). In this immunocompromised patient this represented hyperinfection. Also, fungal hyphae and CMV cytopathologic effects were noted. As expected in hyperinfected immuno-compromised patients, she died shortly thereafter. There was no autopsy. A hemophagocytic syndrome never developed.

PATHOLOGY

Fragments of subcutis contained an atypical infiltrate composed of variably sized lymphoid cells with variable nuclear atypia, nuclear convolutions, cytoplasmic clearing, and scattered mitotic figures. The subcutaneous adipose tissue had been largely replaced. Admixed were numerous histiocytes forming granuloma-like clusters, which sometimes surrounded a vacuoles of apparent necrotic fat. Also, areas of fat necrosis with nuclear karyorrhexis were present. The infiltrate was otherwise relatively homogeneous (i.e., histiocytes and lymphoid cells with no plasma cells or polys). Vessels appeared within normal limits without infiltration or vasculitis. No hemophagocytosis was noted, and special stains (performed at outside hospital) for AFB and fungi were negative. The tumor cells were positive for CD3 (1 to 2+) and CD45RO (3 to 4+); tumor cells were negative for CD20, CD30, lambda, and kappa. An anti-glycophorin stain showed no evidence of hemophagocytosis. Focal positivity was seen with CD56 and CD57, but this positivity may not represent tumor cells. The histiocytes were strongly positive for CD68 and were interpreted as benign reactive cells. Molecular cytogenetic study (i.e., Southern blotting) showed positive T-cell beta-receptor gene rearrangement, which was c/w a clonal or lymphomatous population of T-cells. Immunoglobulin light chain and heavy chain gene rearrangements were not detected. These results are consistent with diagnosis of subcutaneous T-cell lymphoma.

DIAGNOSIS

Subcutaneous panniculitis-like T-cell lymphoma.

DISCUSSION

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare T-cell lymphoma comprising less than ~1% of all non-Hodgkin lymphomas. The tumor develops within subcutaneous tissue and is composed of atypical cytotoxic T-cells of varying size. Often, there is marked tumor necrosis and karyorrhexis. It occurs in males and females with a ratio of 1:1, and patients have a broad age range (i.e., cases have been reported in children under 2 years, but most occur in young adults). Just as in the current case, patients present with multiple subcutaneous nodules, usually in the absence of other sites of disease. Indeed, lymphadenopathy is usually absent. The nodules can be up to ~13 cm in diameter with larger

nodules sometimes becoming necrotic. Common tumor sites are the extremities and trunk. Some patients may have a hemophagocytic syndrome complicated by pancytopenia, fever, and hepatosplenomegaly. SPTCL of the gamma delta phenotype have associated immunosuppression, which appears to be a predisposing factor. But, in most patients the disease presents sporadically; Epstein Barr virus is absent; and there is no known precursor lesion. Initial biopsies the infiltrate may appear deceptively benign, leading to the under diagnosis of malignancy. This tendency is amplified by the frequently present numerous admixed reactive histiocytes, particularly in areas of fat infiltration and destruction. However, the infiltrate extends diffusely through the subcutaneous tissue, often without sparing of septae. In the typical case, the dermis and epidermis are not involved, and this feature is helpful in the differential diagnosis from other lymphomas involving skin and subcutaneous tissue. Yet, SPTCL of gamma delta T-cell origin may show both dermal and epidermal involvement. Another helpful diagnostic clue is the rimming of the neoplastic cells around individual fat cells. Necrosis and karyorrhexis are common, and vascular invasion may be seen in some cases. The neoplastic cells range in size from small cells with round nuclei and inconspicuous nucleoli to larger transformed cells with hyperchromatic nuclei. Tumor cells can have a moderate amount of pale-staining cytoplasm. Classic SPTCL cells have a mature T-cell phenotype, usually CD8-positive, with expression of various cytotoxic molecules including granzyme B, perforin, and T-cell intracellular antigen (TIA-1). Most cases are derived from $\alpha\beta$ cells, although 25% of cases may be $\gamma\delta$ positive. Cases of $\gamma\delta$ origin are often double-negative for CD4 and CD8, and positive for CD56. The probable normal counterpart is the mature cytotoxic T-cell. Tumor cells show rearrangement of T-cell receptor genes, and they are negative for Epstein Barr viral sequences. Thus far, no specific cytogenetic features are known. SPCTL is aggressive, but patients usually respond to combination chemotherapy. Dissemination to lymph nodes and other organs is uncommon and occurs late in the clinical course. A frequent complication is the hemophagocytic syndrome, which often causes a fulminant downhill course. However, the hemophagocytic syndrome may remit following chemotherapy. The differential diagnosis of SPTCL primarily includes various benign panniculitides (e.g., erythema nodosum, erythema induratum, nodular vasculitis, infection, etc.) and other T-cell lymphomas involving the skin (e.g., adult T-cell leukemia/lymphoma, angiocentric N/K-T-cell lymphoma, and cutaneous CD30 positive lymphoproliferative disorders).

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QUESTIONS

1. Subcutaneous panniculitis-like T-cell lymphoma is sometimes associated with a hemolytic-anemic syndrome. **True or False.** _____.
2. Vasculitis is usually present in subcutaneous panniculitis-like T-cell lymphoma. **True or False.** _____.

1. Il linfoma sottocutaneo a cellule –T "panniculitis-like" è a volte associato a sindrome anemico-emolitica. **Vero o Falso.** _____.
2. La vasculite è un reperto di solito presente nel linfoma sottocutaneo "pannucilitis like". **Vero o Falso.** _____.